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# NUCLEAR PHENOMENA ASSOCIATED WITH HETEROTHALLISM AND HOMOTHALLISM IN THE ASCOMYCETE NEUROSPORA[1]

By B. O. DODGE

*Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

A new genus of ascomycetes with four species has been recently described by Shear and Dodge (*18*)[2]. The culture work reported in that paper established the connection between the conidial and the ascocarpic stages. It also proved that two of the species, *Neurospora sitophila* and *Neurospora crassa*, are heterothallic. A preliminary study of the nuclei in spore formation in *Neurospora tetrasperma*, which is ordinarily homothallic, suggested that by a proper selection of spores on the basis of their size, monosporous mycelia could be obtained which would produce only conidia when grown separately, but which when properly mated would also develop ascocarps. The present paper discusses the nuclear behavior which accounts for the fact that heterothallic strains may be segregated out of a species which is commonly homothallic.

The ascus of *Neurospora tetrasperma* usually develops only four spores, and each spore contains two nuclei at its origin. The method by which two or more of the eight nuclei in an ascus cooperate in the delimitation of an ascospore is described for the first time.

## MATERIAL AND METHODS

Material for cytological work on *Neurospora tetrasperma* was obtained from cultures on corn-meal agar in test tubes and Petri dishes. If conidia or fragments of mycelium are sowed on corn-meal agar, perithecia will begin to appear about the fifth day, so that material for fixing may be had from cultures which are from 5 to 10 days old, depending on the temperature of the culture room.

Small pieces of agar bearing ascocarps were fixed in Flemming's weaker solution. Sections were cut 5 microns thick and stained with the triple stain. The perithecia usually form on the surface of the agar, so that orientation of the fruit bodies in cutting is not difficult. Not infrequently the perithecia develop below the surface of the agar. Such material is not as satisfactory for this work. The perithecia of *Neurospora crassa* do not fix as well as do those of *N. sitophila* and *N. tetrasperma*. Only those results obtained by a study of the last species are given in this paper.

[1] Received for publication April 19, 1927; issued October, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 304.

## NUCLEAR BEHAVIOR IN THE ASCUS OF NEUROSPORA TETRASPERMA

The ascus crosier appears to be formed in the usual fashion and later the two nuclei in the young ascus fuse. In this stage it is usually seen that the cytoplasm of the ascus has been differentiated into two regions. The central part, more finely granular, is to become the spore plasm. The epiplasm in the upper and lower ends of the ascus contains large vacuoles which are somewhat elongated.

After nuclear fusion has taken place and the young ascus has elongated, the development of a thickening at the apex of the ascus goes on until it can be recognized clearly as a red ring when stained with safranin. In fixed material the cytoplasm at both ends of the ascus seems inclined to shrink more than the central portion, perhaps because it is more vacuolar. The fusion nucleus is often surrounded by several so-called extranuclear bodies which stain deeply (pl. 1, A).

The spindle of the first division lies along the long axis of the ascus (pl. 1, B). In no case has a transverse spindle been found at this stage although the ascus is rather broad and there would be sufficient space for a transverse spindle.

After the first division is completed the reorganized daughter nuclei, moving apart, come to rest in various positions. They may be widely separated, one lying in the upper, the other in the lower part of the spore plasm. In that case the spindles of the second division will appear to be nearly longitudinal (pl. 1, D). This material gives an abundance of the two-nucleate stage, but the chromatin does not differentiate well in staining. The chromosomes in the metaphase stages of the first and second divisions are very distinct so that they might be counted without great difficulty. If the daughter nuclei had come to rest in the position shown in Plate 1, C, the two spindles would then be oriented in some such way as are those shown in Plate 1, E and F. At E the remains of the old

### EXPLANATORY LEGEND FOR PLATE 1[1]

A.—Primary nucleus with extranuclear bodies. Cytoplasm differentiated into the central spore plasm, and epiplasm at each end with large vacuoles. Shrinkage during fixation at the upper and lower ends.

B.—Metaphase of the first division. The spindle is parallel to the long axis of the ascus. The pointed ends of the spindle take the safranin stain readily.

C.—One type of the two-nucleate stage where the nuclei are not oriented on the central longitudinal axis of the ascus; large vacuoles in either end of the ascus.

D.—Second division in metaphase stages. The spindles are longitudinal, one somewhat above the other but at a different focus.

E.—Another type of division in which the spindles lie oblique to the walls of the ascus. The remains of the old nuclear membrane somewhat collapsed, show distinctly on both of the spindles. Very little cytoplasm in either end of the ascus; spore plasm rather dense and finely granular.

F.—Late telophase stage of the second division. Spindles oblique, one somewhat above and parallel to the other. The polar chromatin masses connected by filamentous structures which take the stain rather heavily. Remains of the parent nuclear material seen at the center of the upper spindle.

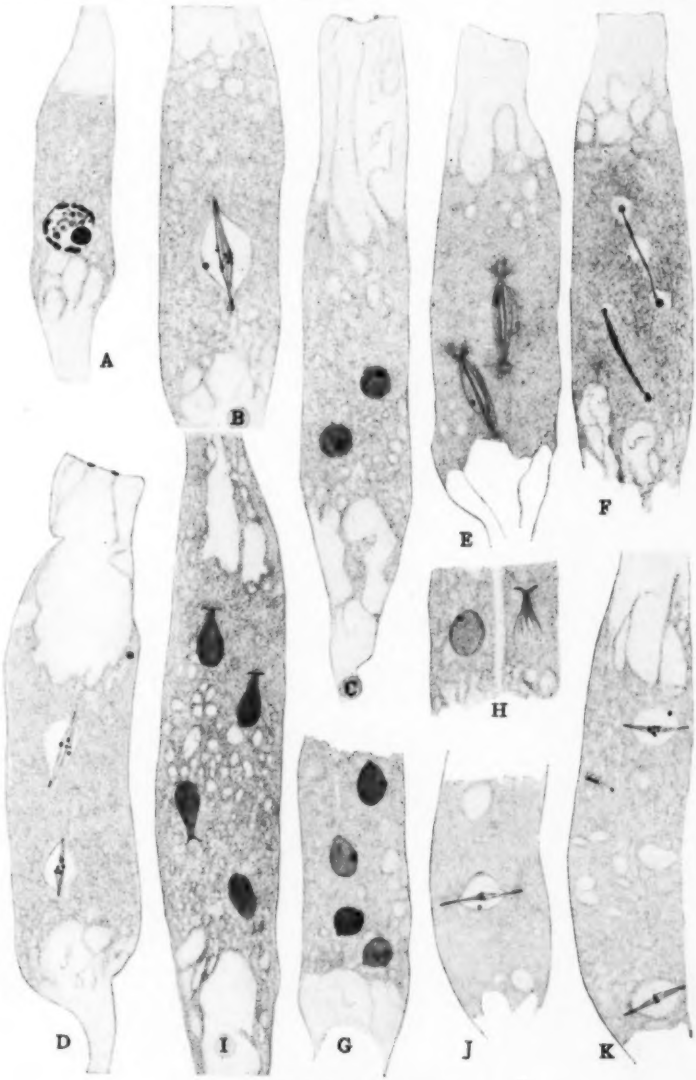
G.—Four-nucleate stage, the pair of nuclei lying at a higher focus, more heavily shaded. Such a distribution of the nuclei may well result from divisions similar to those shown in Figures E and F. The two upper nuclei are somewhat pear shaped. The relationship of the nuclei can be determined by the location of the central bodies; compare with I.

H.—Two views of the same nucleus somewhat more enlarged than in the other figures. At the left and seen at a higher focus, the nucleus appears nearly spherical or without particular distortion. At the right, part of the same nucleus, seen at a lower focus, now shows the beak-like cap with forked appendages.

I.—Four-nucleate stage in resting condition. Nucleoles distinct. Each nucleus is pear shaped or crowned by a beak-like cap of fibers at the end of which is a forked appendage. The beaks of the sister nuclei extend in opposite directions. The spore plasm at this stage is divided into two parts by a series of central vacuoles.

J and K.—Two sections of the same ascus during metaphase stages of the third division. J should have been mounted so that the nucleus would have been at about the same level as the lower nucleus in K. At the upper end of the ascus in K can be seen one complete spindle and the tip end of a second, the remainder of which appeared in the next section and was not drawn.

[1] The writer is indebted to Lulu O. Gaiser for assistance in drawing a number of the figures included in the plates.

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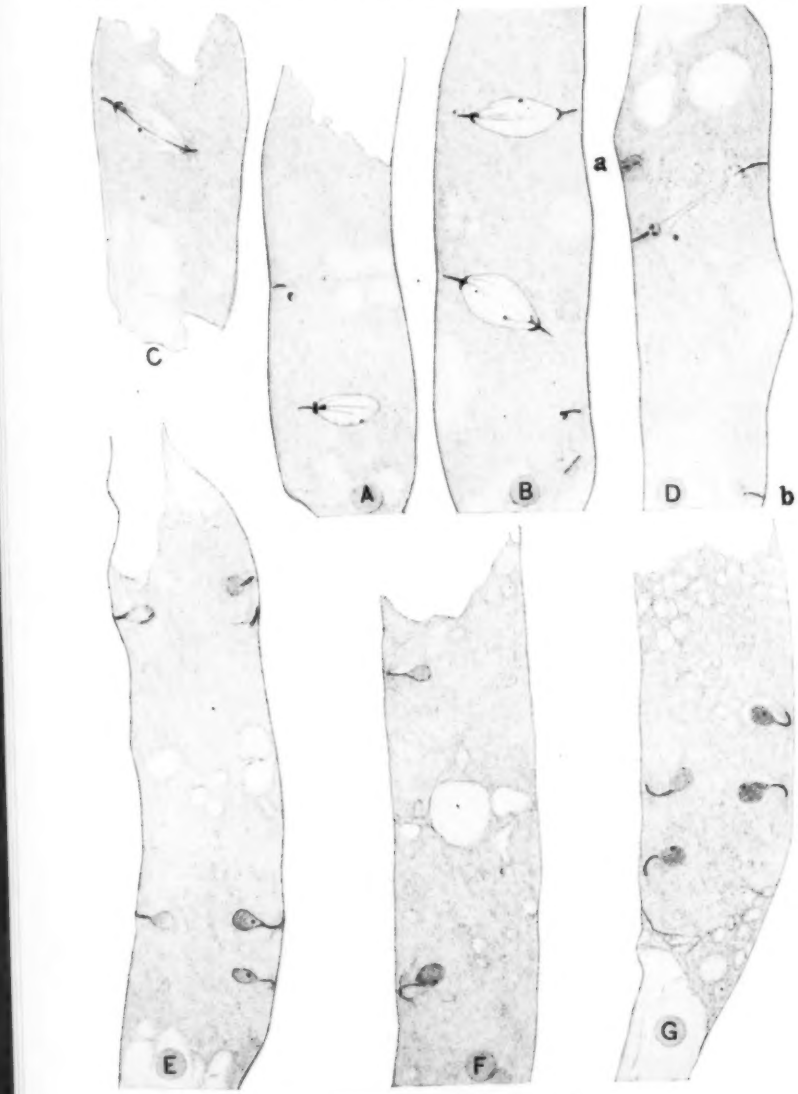


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nuclear membranes and the fibrous material connecting the sister polar masses are still visible. Slightly later stages are shown in F. The daughter nuclei in each pair are beginning to reorganize while the darkly staining connecting strands are still very prominent.

In about half of the figures of the second division catalogued the spindles present the appearance of conjugate division. They lie more or less parallel to each other and oblique to the long axis of the ascus. Divisions in the ascus have heretofore been regarded merely as simultaneous. Conjugate divisions are devices to insure nonsister nuclei being brought together in a common cytoplasm. With such a method of division as is shown in Plate 1, E and F, each end of the ascus would surely contain a pair of nonsister nuclei. If such an arrangement serves a useful purpose, and it would if segregation of the sex factors occurs in the first division, how can such a contingency be provided for in case the dividing nuclei are located as shown in Plate 1, D? Fully 50 per cent of the division stages at this time actually show these spindles rather widely separated. This would suggest that after the second division there would of necessity be a pair of sister nuclei in each end of the spore plasm all in one row. The writer has never seen such an orientation in *Neurospora tetrasperma*. Since they do not lie on the same axis of the ascus, a shifting of position of the reorganizing nuclei, still connected by fibers, must take place, so that a pair of nonsister nuclei will come to lie in each end of the ascus. This is similar to the shifting of pairs of nuclei in the promycelium described by Dodge and Gaiser (*5*) for *Caeoma nitens*, except in a reverse order and with the opposite effect.

It will be shown later in the discussion that perhaps nothing in the cytology of the ascus is more important in its bearing on the experimental results obtained from growing ascospores and crossing different species of Neurospora, than is a knowledge of the genetic relationship of the two nuclei which one sees in each end of the ascus after the second division. Practically any section of a perithecium in which the asci are developing vigorously will show such figures as are given in Plate 1, I. Because of the beautiful symmetry presented in such cases there can be no doubt that the nuclei whose beaks extend in opposite directions are sister nuclei.

Each of the four nuclei shown in Plate 1, G, is slightly elongated. A central body is clearly evident at the point of greatest elongation of each nucleus—that is, on the side toward the top of the figure for the two upper nuclei and on the side toward the bottom of the figure for the two lower ones. The nuclei that are more heavily shaded

EXPLANATORY LEGEND FOR PLATE 2

A to C.—Three consecutive sections of the same ascus. The first section, A, shows a large part of one spindle and a part of one spike-like appendage of another. The rest of these nuclei appear at the lower end of the ascus, shown in B. The chromatin material is bunched at each end of the spindles and spike-like prolongations somewhat curved, extend out to the plasm membrane. The two nuclei shown in the upper end of the ascus, B and C, are members of a pair dividing conjugately, one almost directly under the other but more obliquely placed.

D.—Slightly older or late telophase stage. The daughter nuclei nearly reorganized and showing the curved spiked extensions now pressing against the plasma membrane on each side.

E and F.—Two adjacent sections of the same ascus showing the eight nuclei at the beginning of spore formation. The nuclei clearly in pairs, symmetrically placed, two on one side and two on the other in each end of the ascus. The appendages are now strongly curved and from these structures are proceeding very delicate astral rays. The spore plasm of the ascus is divided into two regions by large vacuoles arranged across the ascus at about the middle.

G.—Stage in spore formation in one end of an ascus somewhat later than the stages shown in E and F. Each spore will contain a pair of nonsister nuclei from the beginning.

were at a higher focus. They are undoubtedly sister nuclei. The best evidence for this assumption is that the central bodies are symmetrically located on opposite sides of the nuclei. Viewed at right angles to the plane of the section shown in Plate 1, G, such a disposition of the four nuclei could well have followed from an orientation of the spindles like that shown in E.

The writer has described the nuclei at this stage as pear shaped or as having long beaks (pl. 1, I). When, however, one studies such sections as are illustrated in Plate 1, H, or those of more delicately stained preparations, the beak sometimes appears to be made up of a cone of fine fibers capping the nucleus, which perhaps is only slightly elongated. In spite of this evidence the writer is not convinced that such figures represent early stages in the reorganizing of the daughter nuclei.

Another reason why Plate 1, I, is assumed to represent a stage long after the second division, is that the spore plasm is now very definitely divided into two sections by a series of vacuoles extending somewhat obliquely across the ascus. These vacuoles enlarge and run together more and more after the third division (pl. 2, E and F.) The organization of the fine granular material at the poles (pl. 1, E) suggests slightly the origin of the forked appendages, yet at the poles of the spindle in F there is not the slightest indication of such structures. Plate 1, F, clearly represents a later stage than E.

A study of slightly later and more critical stages may show that the antlerlike structures, central bodies(?), attached to the chromosomes by fibres, simply break in two and move straight out to the walls of the ascus on opposite sides, giving metaphase stages like those shown in Plate 1, J and K.

In the third division the spindles are transverse—that is, perpendicular to the ascus walls. In the metaphases of all three divisions one sees at each end of the spindles a sharply pointed mass of deeply staining substances, a part of which is undoubtedly the centrosome, although astral rays do not show at all prominently at any stage in this material.

Usually not all of the four spindles of the third division lie in one plane. The section may show a longitudinal view of the upper spindle, while the lower spindle appears only in a polar view. The sections for the illustrations for Plate 1, J and K, and Plate 2, A and C, were chosen particularly because, showing longitudinal and not polar views of the spindles, they give the best idea of the relationship of the eight nuclei resulting from this set of divisions.

In the telophase stages the now curved and more densely staining end structures appear to be attached to the ascus walls, the chromatin bodies being bunched together in two or three masses (pl. 2, A, B, and C) and the remains of the old spindle still stretching across the ascus. A still later stage is shown in Plate 2, D, in which the reorganization of the daughter nuclei is about completed.

The next stage shows pairs of small nuclei side by side, pressing against the ascus wall. Symmetrically placed on the opposite side of the ascus, is another pair of similar nuclei (pl. 2, E and F). Seldom are all four nuclei seen in one section. Serial sections, however, invariably show the nuclei which were missed in the preceding section.

The beaks of the nuclei now become further prolonged and curved sharply at the ends like umbrella handles. Astral rays are very

delicate in these species so that the details in spore delimitation are not adequately brought out. There can be no doubt, however, as to the nature of the process or of the fact that adjacent nonsister nuclei cooperate in cutting out the spores. In the early stages it is sometimes impossible to tell whether one very large spore or two of normal size will be delimited in one end of an ascus, especially in cases where four nuclei lie in nearly the same plane (pl. 2, G). If the plasma membrane and vacuoles take part in the process, it is only in a secondary way. Large vacuoles often appear both within and outside the spore opposite the nuclei (pl. 3, A and B).

There must be much twisting, turning, and slipping of the spores just as soon as they are fully cut out; otherwise they would not be uniseriate at maturity, and such sections as are shown in Plate 3, A, in which the two nuclei in each of the four spores all point in the same direction, would not be found. Three such cases were observed in this material. The nuclei in adjacent spores usually extend in opposite directions (pl. 3, B, above). The curved tip ends of the beaks must be fairly definite structures (pl. 3, C). With delimitation complete, the body of the nucleus becomes detached from the curved end (pl. 3, D) and moves down to the center of the spore (pl. 3, E). After the four spores have enlarged somewhat and become more or less elliptical, their nuclei divide simultaneously. The spindles in a spore usually are not in the same plane. Parts of two adjacent sections of the same ascus are shown in Plate 3, F and G. The spindles of the spore at *a* and *a'* are at right angles. The mature normal spore will have four nuclei (pl. 3, H).

## SIGNIFICANCE OF ABNORMAL SPORES

The most interesting phase of spore formation is brought out in Plate 3, B, where the formation of four spores, each with a single nucleus, is going on in one end of an ascus while two spores with two nuclei each are being cut out in the other end. The genetic relationship of the nuclei in the uninucleate spores can be told by the position of the beaks, which extend in opposite directions when the nuclei are sisters. The two adjacent spores at the right of Plate 3, B, as well as the two at the left would have been of opposite sex; but it is problematical where they would finally lie in the mature ascus. In a narrow ascus, such as is found in *Neurospora crassa* and *N. sitophila*, the two spores developed from sister nuclei are always adjacent in the mature ascus. Further work is necessary to learn the details of the processes by which more than two nuclei cooperate in spore formation in *N. tetrasperma*. As noted previously, an ascus of this species normally contains four spores (fig. 1, A). If one crushes four or five perithecia in which asci are maturing, he will probably find one or two asci with an abnormal number of spores (fig. 1, B to E); most rarely of all, an ascus may contain only one giant spore. If the nuclei of one sex be indicated in black and those of the other sex in white, Figure 1, F and G, will represent the way in which the nuclei cooperate in cutting out spores with two nuclei, and also the way in which they sometimes fail to cooperate, with the result that small uninucleate, unisexual spores are developed. Figure 1, H and I, show how either four or eight nuclei could act together in cutting out large spores. This point has not been followed up.

Shear and Dodge (*18*) found that monosporous mycelia from small spores develop large numbers of sclerotia or bodies which resemble aborted perithecia. No ascospores are developed in such structures. By properly mating these cultures, normal perithecia are formed with asci which have commonly only four spores. The larger, normal spores, have two nuclei when first delimited and thus contain nuclei of both sexes.

These conditions are in an entirely different category from those described by Burger (*3*). Cunninghamella is heterothallic. Burger reports isolation of monosporous mycelia which he says may be called hermaphroditic. Work with these aberrant mycelia was entirely discontinued so that the cultures were lost and this work has not been repeated or confirmed. What little is known of the cytology of the Mucoraceae would suggest that Burger may well have been correct in his conclusions. The condition described might depend on where and how completely segregation takes place, and the chances for the accidental inclusion of nuclei of both sexes in the same spore at some critical point following the germination of the zygospore. Such an explanation, however, is discounted by Burger, who is inclined to entertain the idea of compatibility, although he has no explanation to offer for the condition which he describes.

The writer refers to monosporous mycelia of the heterothallic species as haplonts and uses the terms "unisexual," "bisexual," "sex," and "sexuality" in this paper without knowing, in fact, whether or not the mycelia of the species of Neurospora produce functional oogonia and antheridia. Both reciprocal haplonts produce coiled structures which enter into the development of the perithecium. Further study will no doubt demonstrate that these primordia are morphologically and functionally different. Work on crossing of the haplonts of different species, to be reported later, shows that the descendants of nuclei from both haplonts must finally fuse in the ascus.

Knowing that the existence of "psuedo-heterothallism" in *Neurospora tetrasperma* is based on the accidental inclusion of only one of the two sexually different nuclei which are normally contained in each spore at its origin, a way is suggested by which one may be able to obtain homothallic strains from heterothallic species, such as *N. crassa* and *N. sitophila*. The former species particularly may,

EXPLANATORY LEGEND FOR PLATE 3

A.—Section of an ascus showing four spores just after they have been completely delimited. Each spore contains a pair of nuclei close together. In this case the spores have readjusted their positions so the beaks in the four pairs all extend in the same general direction. The epiplasm is considerably shrunken from fixation and the spores have not as yet assumed their final elliptical shape. Very fine astral rays are still visible proceeding from the curved projections of the nuclei.

B.—Ascus showing the basis for the development of homothallic and heterothallic spores in the same ascus. Each of the two spores in the upper end of the ascus contains two nuclei of opposite sex. Each of the four spores in the lower end of the ascus contains only a single nucleus. Since the nuclear beaks of the two spores at the right extend in the same direction, they are probably of opposite sexes. Each spore of the pair at the left is also unisexual. The spores with one nucleus are much smaller than those containing two.

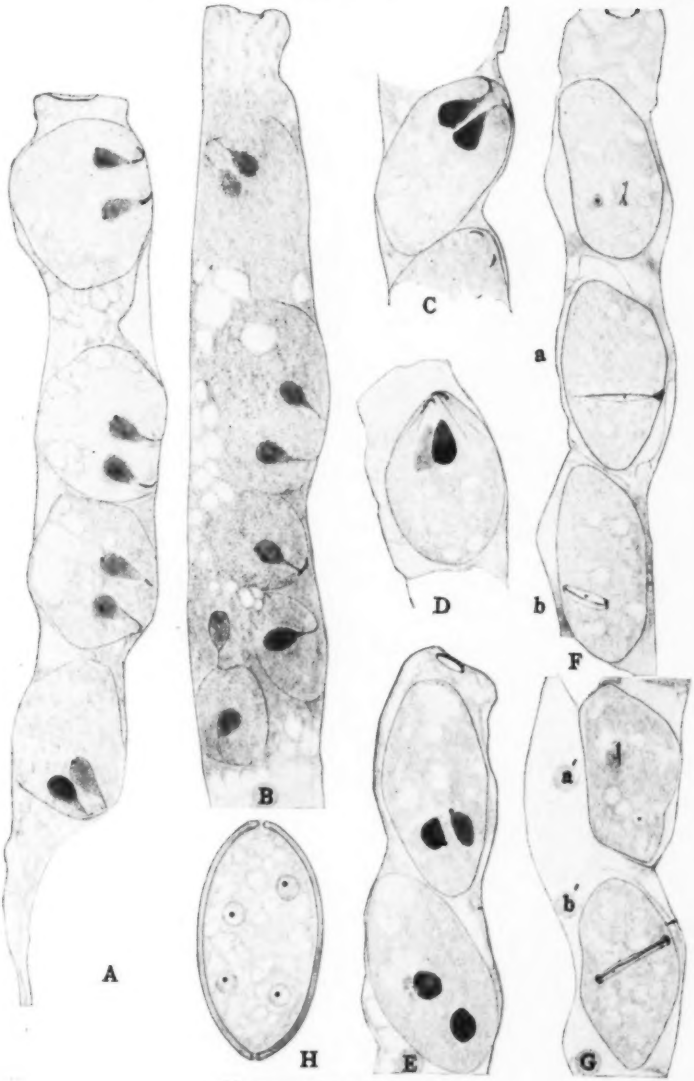
C.—Later stage in spore formation. The nuclei about to break away from the tip ends of the curved beaks. In the lower of the two spores, the appendages which were cut off in sectioning are indicated at the right.

D.—Still later stage in spore formation. The nuclei have been completely detached from the appendages, which are still visible at the upper end of the spore.

E.—The daughter nuclei have become nearly rounded up and the spores no longer show the remains of the hooked nuclear beaks.

F and G.—Parts of two sections of the same ascus showing dividing nuclei in telophase stage. At a and a′ are shown two sections of the same spore, and at b and b′ two sections of the next lower spore. Two nuclei in each of the four spores of this ascus were all in the same stage of division

H.—A mature spore after reorganization of the four daughter nuclei and the thickening of the spore wall; a germ pore is visible at each end

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on rare occasions, develop an ascospore which is much too large for a spore with only one nucleus at its origin (fig. 1) and can not be accounted for as simply a matter of competition for the nourishment in the spore plasm. Should such a spore contain two or more nuclei at its origin (fig. 1, G, H) and have been delimited in the same way as are the normal spores of *N. tetrasperma*, then, on germination, a homothallic mycelium will be developed. Clearly heterothallism and homothallism in the species of Neurospora are not absolutely fixed specific characters, although the sexual nature of an individual haplont is definitely determined by the time the spore is cut out.

The occurrence of spores of different sizes in the ascus of *Bulgaria inquinans* has long been a matter for speculation. Moreau (*14*) finds that some spores are large and others are small for two very different reasons. All of the nuclei do not necessarily divide simultaneously so that one may sometimes find resting nuclei of both the second and third generations in the ascus at the same time. A nucleus of the second generation will be much larger than one of the third. Spores formed at once and including nuclei of the third division, this author believes, having the first chance at the nourishment in the spore plasm, would become larger than the other spores formed in the same ascus later. Regardless of the size of the spore, each one contains only one nucleus at its origin. When two of the original eight nuclei are included in the same spore, as Wolf (*20*) found in *Podospora anserina*, the spore will be much larger than one with only a single nucleus. Such forms as *P. anserina* and *Bulgaria polymorpha* should prove extremely interesting. Has the former a heterothallic sister species? May not the smaller uninucleate spores of the latter be unisexual and the larger binucleate spores be bisexual as they are in *Neurospora tetrasperma?* Faull (*6*) figures a two nucleate spore of Neotiella, and Fraser (*9*) shows a spore of *Humaria rutilans* containing five nuclei at its origin. These authors considered such spores simply as abnormal and of no consequence. The writer has pointed out above the significance of the inclusion of different numbers of nuclei in spores, particularly in cases where heterothallism must be taken into account.

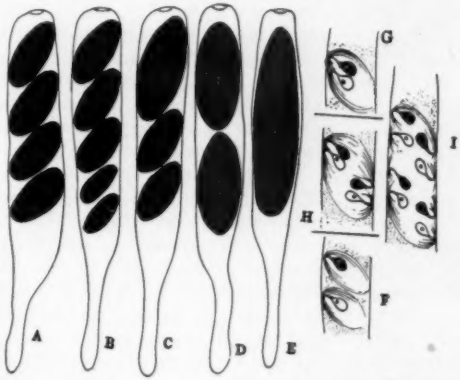


FIG. 1.—Diagram of asci with various numbers of spores at maturity and method of spore delimitation. A, normal number of spores is four, very rarely only one giant spore (E) is formed; F, small uninucleate, unisexual spores; G, bisexual, binucleate spores; H, bisexual four-nucleate spore; I, eight-nucleate spore in which all of the nuclei resulting from the third division of the fusion nucleus cooperate in cutting out the spore. H and I theoretical

## HETEROTHALLISM NOT DIOECISM

Perhaps 50 per cent of the red algae are heterothallic. In the filamentous and thalloid green and brown algae the male and female

gametes of certain species are borne on different plants. The same is true for liverworts, mosses, and ferns; and, of course, all seed plants are heterothallic. Among the fungi, certain species of water molds have long been known to be heterothallic. Thanks particularly to Thaxter, one order of ascomycetes alone, the Laboulbeniales, now presents at least a dozen genera with some hundred species which are heterothallic. In all of the groups mentioned, it is readily demonstrated that some gametophytes are male and others are female, although the cytological details of nuclear behavior may be entirely unknown. With such evidence at hand, it is strange that the existence of heterothallism among our higher ascomycetes should have been so long unknown.

Blakeslee (*2*), when he first proposed the use of the terms, stated that "heterothallic" and "homothallic" correspond to "dioecious" and "monoecious" in the flowering plants. In all his subsequent writings this author shows that originally he must have intended the illustration merely as an analogy. Allen (*1*) working on the genetics of Sphaerocarpos has emphasized the importance of keeping in mind the difference between male and female plants of the liverworts, mosses, etc., on the one hand, and staminate and pistillate individuals of the flowering plants on the other. Pollen tubes are male haplonts, embryo sacs are female haplonts. Recently Sharp (*17*) in discussing the factorial interpretation of sex determination rightly insists that all flowering plants are heterothallic. Only a comparatively small number are dioecious. Less confusion might result if, for example, such genera as Amorphomyces and Herpomyces were referred to as heterothallic instead of as dioecious. The terms now have entirely different meanings.

The Mucoraceae apparently do not afford the best material for cytological study. No one has been able so far to find any connection between nuclear behavior and the segregation of the factors determining sexual reproduction in heterothallic forms.

In the case of certain higher basidiomycetes it is now known just what the nuclei do, step by step, from the time the spores germinate until the new generation of spores is formed on the basidium. No more complete story of nuclear behavior is known in the plant kingdom, but there is nothing in the cytology which serves as a basis for predicting which species of Coprinus, for example, will be heterothallic and which ones homothallic.

*Melanospora destruens* (*18*) resembles *Neurospora sitophila* in several morphological features. It is not yet clear why the former should be homothallic and the latter heterothallic. Within the genus Neurospora, however, the factors which determine certain types of sexual reaction are clearly segregated before, instead of after, spore formation, so that a study of nuclear behavior in the ascus enables one to see why one species is heterothallic while another is homothallic.

## SEGREGATION OF SEX FACTORS

In Pyronema the antheridia are developed on dichotomously branched hyphae which are quite distinct from those that build the oogonia. Monosporous mycelia of this species are said (*4*) to produce ascocarps in culture. Each ascospore is at first uninucleate. The segregation of the sexes must therefore take place a long time after

spore germination. A number of ascomycetes outside of the Laboulbeniales develop sex organs which are morphologically and functionally of two different sorts. Since the two spores in an ascus of *Phyllactinia corylea* are formed from sister nuclei (*11*) the chances, one might think, for this species to be heterothallic are small. Yet if the factors for sex differentiation are segregated out in the third division, as may well be the case, spores of two different sexes will then be formed in the same ascus. The production of perithecia by certain species of the Erysiphaceae is very erratic. When culture work on such forms is carried out with monosporous haplonts, no doubt some of these species, such as the mildew on raspberries, will likely be found to be heterothallic.

Thaxter's highly interesting accounts of the development of various species of the Laboulbeniales show that in the heterothallic ("dioecious") forms the spores are of two sorts, usually somewhat different in size. The male spore is frequently somewhat smaller than the female. They are discharged from the asci in pairs and become attached to the host side by side. The sex of each spore has been determined absolutely by the time it has been delimited. In this connection Thaxter remarks (*19, v. 13, p. 222*): "The cytological phenomena * * * which lead up to the sexual differentiation and pairing in the ascus of these male and female spores, is likely to prove of unusual interest." It is a curious fact that in cases where the spores of the hermaphroditic species are frequently discharged in pairs and become attached to the host side by side, only one of the spores of the pair develops into a mature plant (*19, v. 14*). The other spore degenerates without germination.

Thaxter (*19, v. 12, p. 217–218*) further says: "It has been previously mentioned that in a majority of forms [hermaphroditic species] the antheridial appendage is developed from the terminal cell of the germinating spore. The female organs, however, are always formed from the products of the division of the basal cell, never in any case from the terminal cell, where this is present. Although the products of the division of the terminal cell are invariably sterile or male, it is not true, as might be supposed, that the basal cell or its derivatives have any inherent female character, since in many cases both normal and abnormal antheridia and antheridial branches may arise below the point of insertion of the female organ, or even, in exceptional cases, replace it entirely."

This may be the correct interpretation. It may also be claimed that the facts cited go to show conclusively that usually the terminal cell is in fact inherently male and the basal cell female, and that segregation of the sexes takes place during the fourth nuclear division after nuclear fusion, that is, during the first nuclear division after spores have been cut out. Occasionally, however, such a sex segregation is not consummated at this time and antheridial appendages develop from the cell which, normally or abnormally, gives rise to the carpogenic structures. This could very well be due to a failure to lay down the septum until after a second nuclear division in the spore, and the inclusion of one of the extra male nuclei in the basal cell. If the development of the male structures from the terminal cell and the female structures from the basal cell could be reversed

by inverting the spore or in any other way, this would prove, of course, that there is nothing inherent sexually in either segment of the spore.

Thaxter has been unable to determine whether or not the asci of the genus Herpomyces are eight-spored. "If this were actually the case," he says, "it would involve the curious phenomenon of absolute sex differentiation in the last mitosis, which would not necessarily occur in a four-spored ascus" (*19, v. 13, p. 222*).

It is to be regretted that the only cytological work so far published on the Laboulbeniales has been upon two species in which the antheridial structures are said to be unknown, so that sexual reproduction as such would clearly be out of the question. Faull (*8*) finds that in *Laboulbenia chaetophora* the spindles of the first and second mitoses are longitudinal. In the four-nucleate stage the nuclei are in pairs, two in the upper part of the ascus and two in the lower; again in the third division the spindles are longitudinal. After reorganization, four nuclei move to the upper part of the ascus and disintegrate. The four which develop at the lower ends of the spindles take a central position. Faull says further (*7*): "The functional nuclei, in *Laboulbenia chaetophora* at least, are the lower ones in the spindles of the last mitosis. There are some reasons for believing that the same is true of Amorphomyces and Dioichomyces, in which case sexual differentiation of the spores might be determined in the second division."

Assuming that nuclear behavior during the processes leading up to the delimitation of the ascospores should be the same for the heterothallic genera as that given by Faull for the two parthenogenetic species which he studied, is it necessary to assume also that the segregation of the sex factors must occur in the second division? For practically all the species of this group where fecundation is an essential factor for the full development of the ascus, all that would be necessary to make a species heterothallic would be to move the point of segregation back from the fourth to the third nuclear division. Evidently many of the species commonly homothallic have provided for just such an emergency by discharging the spores in pairs.

The features in the cytology of the ascus discussed by previous authors and which have a particular bearing on segregation of the sex factors and other work covered in this paper are: (1) The position and direction taken by the spindles during each of the three nuclear divisions; (2) the location of the resting nuclei in the spore plasm and their relationship, one to the other, at different stages in the development of the ascus; and (3) the part taken by the nuclei during spore formation and the method of spore delimitation. It may be noted that on certain points there is a general agreement among previous writers.

The spindle in the first division of the primary nucleus usually lies parallel to the long axis of the ascus. Harper (*10*) and Claussen (*4*) have pointed out that in Pyronema this spindle may also be transverse. The writer has found that in *Ascobolus winteri* the first spindle is always transverse. One or two other exceptions have been reported.

After the first division has been completed, the two daughter nuclei separate and come to lie one above the other, either on the

median long axis of the ascus, or, just as frequently, on some line which is oblique to the ascus walls. Reports of finding the nuclei lying side by side as though reorganized at the ends of a transverse spindle have not been seen in the literature.

The two spindles of the second division are usually described as lying along the general direction of the ascus axis. In Pyronema, according to Claussen (*4*), and in Phyllactinia, according to Harper (*11*), one of the two spindles of the second division may be transverse. As a general rule when an ascus is long and narrow the four nuclei, following a second division, lie in a row on the long axis of the ascus. The two nuclei in each end of the axis of the ascus would then be sister nuclei. The disposition of the four nuclei must depend largely on how much of the cytoplasm of the axis is differentiated into spore plasm. Jolivette (*16*) says that if a transverse section of the ascus were made after the second division in *Geoglossum glabrum*, the four nuclei would be seen in the same plane. In a longitudinal section two nuclei are seen in one focus and two in the other. The spindles of the third division show no tendency to lie in the transverse axis of the asci.

There are a few other reports of the lack of uniformity in the disposition of the spindles in the third division. Faull (*7*, *8*) claims that all four of the spindles of the third division in Laboulbenia are longitudinal. In most other ascomycetes, however, they are reported to be more or less transverse. Komarnitzky (*12*) and Faull have both remarked on the possible connection or bearing which the genetic relationship of particular nuclei may have on "dioecism" in the Laboulbeniaceae. Their work will be considered further in the general discussion. In most ascomycetes division figures are so rarely found that one should not draw conclusions as to the significance of the orientation of the spindles in certain cases in which it is clear that the author has based his report on only one or two spindles which he was fortunate enough to find.

The first spindle in *Verpa bohemica* (*12*) is nearly longitudinal. The spore plasm is developed in the upper end of the ascus and all of the nuclear divisions occur in this end. Of the two spindles in the second division, the upper appears to be transverse, the lower somewhat oblique. The only spindle shown in the third division is nearly transversely placed. The evidence presented by Komarnitzky's figures is not conclusive as to the particular nuclei which are concerned in spore formation. Two spores are finally cut out and the other six nuclei degenerate. Komarnitzky realizes, however, the importance of knowing the exact orientation of the spindles during the three different divisions in the ascus.

If no switching or exchange of position of the nuclei takes place after their reconstruction, in other words, if one is justified in drawing conclusions as to the genetic relationship of particular nuclei from the position and orientation of the division figures, then in the "dioecious" forms of the Laboulbeniaceae, segregation of the sex factors may very well take place in any one of the three divisions with the same result, provided the nuclei take the positions and the spindles are oriented as Faull (*8*) figures for Laboulbenia. In all three divisions the spindles extend lengthwise of the ascus. Faull (*8*, *fig. 57*) shows that the four nuclei resulting from the second mitosis lie in pairs, two in one end of the ascus and two in the other, comparable

to what occurs in *Neurospora tetrasperma* (pl. 1, I). It will appear in due time, however, that one would be led far astray were he to presume to draw conclusions as to the time of segregation and the sexual nature of the eight spores in the heterothallic species *N. sitophila* from what he finds to be true of nuclear behavior in the homothallic form *N. tetrasperma*.

Full information regarding the early prophase stages as the four nuclei of *Neurospora tetrasperma* go into the third division, has not been obtained. This is a critical stage, not only because of its bearing on the question as to where segregation takes place, but also because further light is desirable as to the nature of the structures which always appear as forked appendages at the beaks of the nuclei at this time. If these horns represent merely lines of flow, or a massing due to fixation of astral rays, one would not expect to find such regularity in their form and position. They certainly appear more like definite organs, in the nature of monstrous central bodies or blepharoplasts.

Harper (*11*) shows that immediately after the first division the two nuclei in Phyllactinia are pear-shaped. He states that they always round up, however, before beginning the second division. The same thing may take place in the case of the four nucleate stage of Neurospora so that the stage shown in Plate 1, I, would precede that shown in Plate 1, G. There is much evidence against such a view, however. First, the two pairs of nuclei undergoing the third division are at opposite ends of the ascus and widely separated. There is practically no change of position after the hornlike appendages have developed. In the stage shown in Plate 1, G, the nuclei are not in the final position for the third division. The beaked nuclei in Plate 1, I, may not be fully mature or reorganized, yet they are certainly in a resting condition and must so endure for a long time. Such stages are very abundant in the material. If the nuclei dispose of the beaks and forked appendages before going into the third division, they must do so in a very short space of time. With the development of the spindle of the last division, each end of it is provided again with a curved spikelike structure which extends out to the plasma membrane. After the first division on the contrary the two nuclei round up and remain in this condition for some time.

Since the factors upon which sexual reproduction in the heterothallic species of Neurospora depends are lodged in two different spores and in two different nuclei of the same spore in homothallic species, it is essential to a proper understanding of the questions involved to determine at what time and in what way the segregation of the sex factors takes place.

Shear and Dodge (*18*) have reported that in *Neurospora crassa* four spores in an ascus are of one sex and four of the other. Nothing is said concerning the position in the ascus of each of the eight spores.

Students of heterothallism of the basidiomycetes, forced to explain some of the curious results of their culture work, are assuming that two pairs of factors, "sex factors," control the reactions leading to the formation of clamp connections. Newton (*15*) believes that in *Coprinus lagopus* segregation of one factor may take place in the

first division in the basidium and the segregation of the other factor in the second division. The writer has not been confronted with such complicated situations so far, yet the results obtained by crossing *Neurospora sitophila* with *N. tetrasperma* show the need of exact knowledge of nuclear behavior through the entire life cycle in both species as well as in the different hybrid generations. It will be shown in the following diagrams that nuclear behavior in the ascus of *N. tetrasperma* is such that normally totipotent or homothallic ascospores will be formed, regardless of whether segregation takes place in the first, second, or third division.

One may take as a type an ascus such as that of *Galactinia succosa* figured by Maire (*13*), which shows very diagrammatically the position of the nuclei and orientation of the spindles generally reported for long asci. If segregation of the sex factors takes place in the first division, then as indicated in the diagram (fig. 2) the four spores in the upper end of the ascus must be all alike, and all of one sex, and the four in the lower end must be of the other sex. If segregation of the sex factors takes place in the second division in a species the spores will alternate in pairs, the first two being of one sex and the next two of the opposite sex. With a knowledge of conditions in the ascus of *N. tetrasperma*, a brief study of nuclear behavior in the ascus of *N. sitophila* has convinced the writer that the spores of the latter species will so alternate in pairs. Should segregation take place in the third division, then the four spores in one end of the ascus will alternate as regards their sex. So it is clear that with no shifting of position of the nuclei, three different pictures will result, depending on the place of segregation.

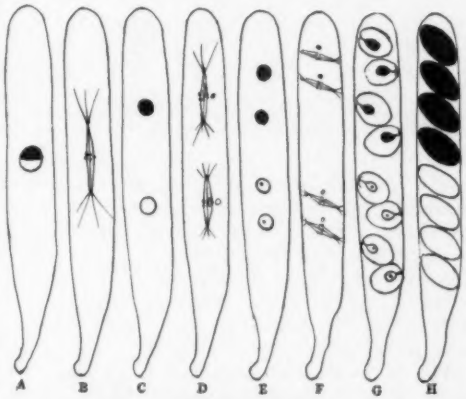


FIG. 2.—Diagram showing nuclear behavior and position of the four spores of each sex in a typical long ascus if segregation of the sex factors should occur in the first mitosis

In *Neurospora crassa* and *N. sitophila*, after the second divisions, the four nuclei lie in a row in the ascus. In no case has the writer found, as previously noted, such a condition in *N. tetrasperma*. The evidence so far discovered indicates that regardless of the position of the two nuclei during the second division in the last species such a shifting must take place as is necessary to bring the four nuclei into the formation shown in Figure 3, E, which is the one picture that seems to be very constant in this material (pl. 1, I).

Should segregation take place in the first mitosis in *Neurospora tetrasperma* the way in which each spore would receive at its origin one nucleus of each sex is indicated in Figure 3, A to H. The spindle of the first division is always longitudinal. The essential difference

between this type of nuclear behavior and that shown in Figure 2 is not in the number of nuclei in the young spore. In a number of ascomycetes with long asci it has been demonstrated, as can be done

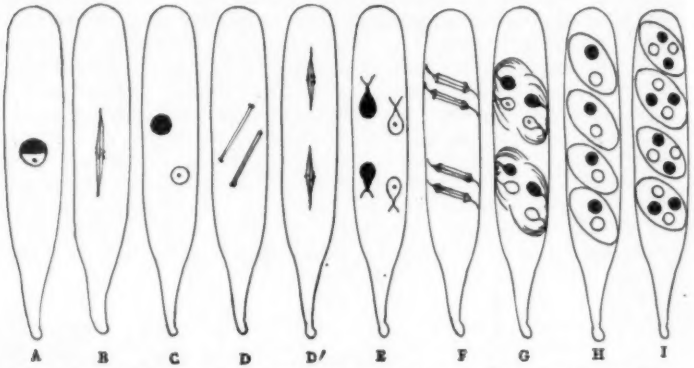


FIG. 3.—Diagram showing nuclear behavior and method of formation of bisexual spores in *Neurospora tetrasperma*. The position of the spindles in the second mitosis may be parallel to each other and oblique to the long axis of the ascus, D, or they may be longitudinal, one in each end of the ascus, D′

easily in case of *N. sitophila* and *N. crassa*, that the two nuclei in each end of the ascus are sisters (fig. 2, E). With segregation taking place in either the second or third division such an arrangement would accord with the experimental results obtained with *N. tetrasperma* should two adjacent nuclei be included in the same spore. Yet the evidence obtained by a study of the shape and the position of the four nuclei of this species after they have come to rest (fig. 3, E) indicates that here, as previously noted, a shifting of the nuclei must occur after divisions like those shown in Plate 2, D, or Figure 3, D′; otherwise fully 50 per cent of the asci at this stage would show four nuclei in a row down the center, which certainly is not the case. No such orientation has been found, although this would account for the occasional formation of large spores which are unisexual.

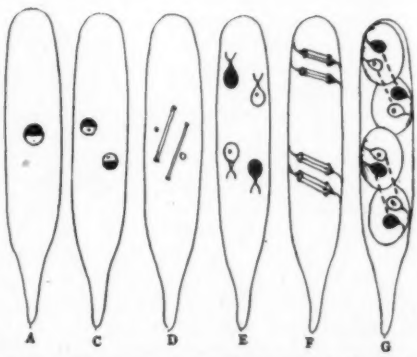


FIG. 4.—Diagram of nuclear condition leading to the development of bisexual spores in *Neurospora tetrasperma*, should segregation of the sex factors occur in the second mitoris, other conditions as in Figure 3

With segregation in the second division in *Neurospora tetrasperma*, the spores will all be bisexual (fig. 4, A to G) no matter in which of the two ways (fig. 3, D or fig. 3, D′) the spindles of the second mitosis are oriented. Occasionally in the case of this species, only one

nucleus is included in each of certain spores (pl. 3, B). With nuclear behavior otherwise the same, such spores would alternate in pairs in the ascus, two being of one sex and two of the other.

Figure 5, A to G, shows how segregation might take place in the third division without preventing the formation of bisexual spores. Perhaps 1 per cent of the monosporous mycelia from what was judged to be either normal spores, or spores which were oversized, failed to develop perithecia. One is forced to find an explanation for this apparent failure in such cases to include nuclei of both sexes in these spores. A section of an ascus which was taken to be abnormal showed what appears to be a pair of nuclei in the second division, with spindles transverse, one in the upper, the other in the lower end of the ascus. Such abnormal conditions are generally said to be of no particular significance, yet these cases may be the very ones to account for unusual conditions, such as the development of large unisexual spores with two or even more nuclei at their origin.

The sexual nature of each of the eight spores in the ascus of *Neurospora sitophila* has been determined culturally by Marguerite Wilcox who has assisted the writer in the preparation of slides for this cytological study. Her report on this work, together with a further account of the cytology of the ascus in this species, is forthcoming. With the cytology of the ascus known and the sexual nature of each of the spores in the ascus of *N. tetrasperma* and *N. sitophila* worked out culturally, the way is cleared for a better interpretation of the results being obtained by crossing reciprocally unisexual haplonts of these two species.

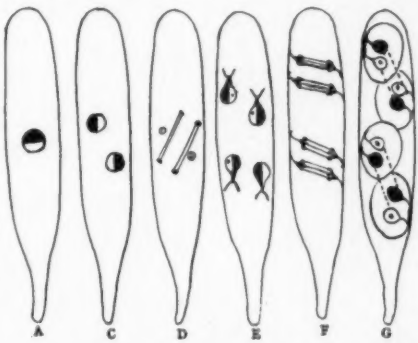


FIG. 5.—Diagram of nuclear condition in an ascus of *Neurospora tetrasperma*, showing the possibility of the formation of bisexual spores, should segregation of the sex factors occur in the third mitosis, other conditions as in Figure 3

## SUMMARY

*Neurospora tetrasperma*, which is normally homothallic, develops asci with four bisexual spores, as contrasted with the heterothallic species, *N. sitophila*, in which the asci are eight spored, and each spore is unisexual. The cytological basis for this difference is reported.

In *Neurospora tetrasperma*, the spindle of the first mitosis is longitudinal; the two daughter nuclei separate and come to rest one somewhat above the other in the ascus.

With respect to the position and orientation of the spindles of the second division two types are described. In the first type the spindles may lie nearly parallel near the center of the ascus, usually somewhat oblique to the long axis, suggesting conjugate division. In the second type, the spindles are longitudinal, one in each end of the ascus.

Each of the four nuclei is pear shaped and the beak is capped by a forked appendage. The nuclei are arranged symmetrically, two nonsister nuclei in each end of the ascus.

The spindles of the third mitosis are nearly transverse. Normally two adjacent nonsister nuclei cooperate in the delimitation of each ascospore through the development of astral rays from their curved beaks. It is shown how, regardless of whether segregation takes place in the first, second, or third division, each spore will contain one nucleus of each sex.

Occasionally adjacent nuclei, for some reason, are not entirely compatible and fail to cooperate in the cutting out of a spore. Each nucleus then acts independently, and as a result uninucleate, unisexual spores, which are comparatively small, are delimited. Very rarely all eight of the nuclei in an ascus cooperate in the delimitation of a spore. Such an ascus will contain only a single giant spore at maturity.

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# THE TWO MOST COMMON DECAYS OF COTTON BOLLS IN THE SOUTHWESTERN STATES[1]

By MICHAEL SHAPOVALOV

*Pathologist, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture*[2]

## INTRODUCTION

It has been frequently reported from cotton-growing sections of the Southwest that immature cotton bolls are subject to a peculiar form of disease to which the name "smut" has been commonly applied by the growers. The writer has made a survey of cotton fields in California and Arizona and in the section of Mexico immedaitely adjoining the Imperial Valley and has obtained a considerable number of cultures from affected specimens. These cultures, as well as subsequent inoculation tests, both in the field and in the laboratory, show very clearly that the disease in question is not a smut. Although it is true that in its final phase of development when the boll has dried up it bears a slight resemblance to smut, its initial stages are plainly those of a soft decay.

This soft-decay form either was overlooked by the average observer or, if noticed, was not correlated with subsequent and more pronounced symptoms. Disease symptoms appear limited to bolls that have previously suffered injury by insects or otherwise.

The writer's studies have also revealed the fact that in reality there occur in the field two forms of the boll decay, both of which generally have been known as "smut." One of these is caused by *Aspergillus niger* Van Tiegh. and the other by *Rhizopus nigricans* Ehr. Although on close examination these forms show very distinct peculiarities throughout the entire cycles of their development, they have nevertheless a certain similarity in their general appearance during the fruiting stages of the causal organism. This similarity undoubtedly is responsible for the confusion of the two troubles, particularly since the decay symptoms formerly had not been recognized.

## ASPERGILLUS NIGER

Although very common on all kinds of decaying vegetable matter, the fungus *Aspergillus niger* has heretofore been associated by investigators with only a few diseases of economic plants. Van Pelt (*10*)[3] considered it to be responsible for a serious black mold of onions in Ohio. Certain laboratory experiments carried on by the writer (*7*) revealed it to be among important potential parasites of the potato tuber. The same fungus has been definitely shown by Phillips and Smith (*6*) to be the cause of the so-called smut of white fig varieties in California.

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[2] The writer expresses appreciation to W. B. Camp, of the Office of Cotton, Rubber, and other Tropical Plants, Bureau of Plant Industry, for the great amount of helpful information given him in regard to cotton culture in the San Joaquin Valley and the Southwest in general, and to H. G. McKeever, of the same office, for invaluable assistance in field observations and the collection of specimens.

[3] Reference is made by number (italic) to "Literature cited," p. 312.

The occurrence of *Aspergillus niger* on cotton bolls was briefly reported by the writer (*8*) in 1925. Aside from the decaying bolls, the fungus was also frequently isolated from young dying squares, from discolored pedicels, and from lesions on the bracts. It was likewise obtained from stem lesions of young seedlings showing symptoms of sore shin. As far as the observations of the last two seasons (1925 and 1926) are concerned, the Aspergillus boll decay was more frequently observed in the Southwest than the Rhizopus decay and was most prevalent in southern portions of California, though specimens of it were found also in the San Joaquin Valley, in Mexican plantations adjacent to the Imperial Valley, and in Arizona.

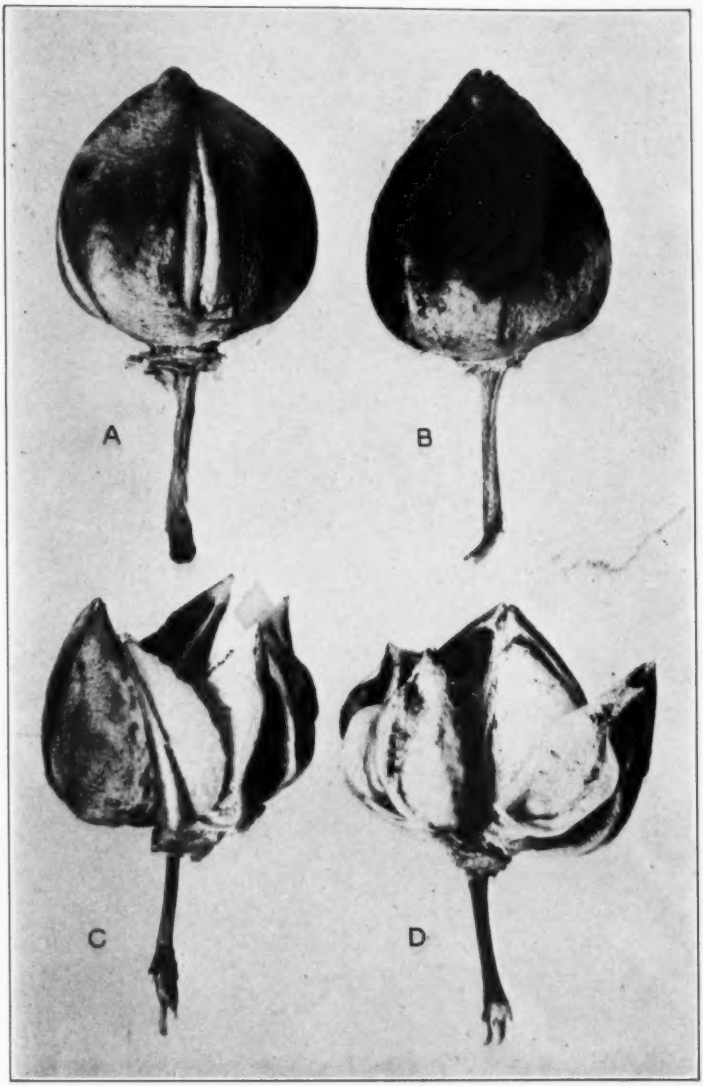
The rot begins as a soft pinkish spot either on the side of the boll or somewhere near its base. The hue and the extent of the pink discoloration vary more or less, but the discoloration is always present and is characteristic of this sort of decay (pl. 1, A). As the lesion increases in size, the color of the older decayed area turns from pink to brown and the original discoloration remains only in the freshly decaying regions—that is, on the border of healthy and diseased tissues. If such a boll is cut open through the affected area the same pink to purplish and red-brown shades of the invaded tissues will be seen (pl. 1, B). The fructification of the fungus begins in relatively early stages of decay, but the spores are white or light gray at first, darken very rapidly, and in a short time give the diseased bolls a "smutty" appearance (pl. 2, B). The fungus is capable of destroying all parts of the boll—the capsule, the lint, and the seeds. Cultures of the organism have been obtained from all such affected tissues. When it is destroyed by the parasite, the entire boll dries up and remains closed (pl. 2, A). Frequently, however, the progress of the decay is checked, either by unfavorable environmental conditions not yet understood, or by the natural development of the boll if it is affected when nearly mature. In such cases the bolls open only partially, the affected portions remaining closed. The black masses of the Aspergillus spores may be seen in abundance both on the inside and the outside of the bolls (pl. 2, C and D).

Artificial inoculations with pure cultures of *Aspergillus niger* were made on growing bolls in the field and in the greenhouse, and on picked bolls incubated in moist chambers in the laboratory. The percentage of infection was greatest when picked bolls were used fresh—as soon as they were brought to the laboratory. Bolls which remained in the room for several days before inoculation gave only a small percentage of infection, showing that a certain degree of succulency of the tissues is necessary for the most destructive results. Atmospheric humidity likewise appears to be essential for the best activity of the parasite. This is shown by the fact that the bolls growing in the greenhouse were affected more readily than those growing in the field. The inoculum was introduced partly by applying it to wounds made either with a scalpel or with a needle, and partly by smearing it on the surface of bolls not injured artificially.

With but one exception—namely, in the case of a group of uninjured inoculated bolls placed in a moist chamber (see Table 1)—positive results were obtained only in those cases in which injured bolls were inoculated. One of the bolls placed in the chamber became infected, but proof was lacking that this boll had been brought from the field entirely uninjured. This seems to indicate that the

**TWO FORMS OF COTTON-BOLL DECAY**

A and B, caused by *Aspergillus niger* Van Tiegh.; C and D, caused by *Rhizopus nigricans* Ehr.
A and C, external appearance of the bolls affected by the two types of decay;
B and D, interior views of the same bolls




Later stages of cotton-boll decay caused by Aspergillus niger Van Tiegh.
A.—Rapidly decayed and dried-up boll with a very scanty fructification of the fungus.
B.—Boll destroyed by a slower decay with abundant fructification.
C. and D.—Partially decayed and partially opened bolls (bracts removed to show larger surface of the bolls).

fungus is mainly if not exclusively a wound parasite. The decay as it naturally occurs in the field often is associated with visible insect wounds, particularly with those made by the bollworm, although in many instances such injuries are not apparent. It is possible, however, that the infection in these cases is associated with some minute punctures caused by smaller insects. In the case of the wound inoculations, the decay did not always appear on the surface at the point of the inoculation, but sometimes developed first within the boll and then broke through at a point more or less removed from the wound. The time necessary for the appearance of the first external signs of the decomposition varied from three to eight days.

A more detailed account of the inoculation tests and their results is given in Table 1.

TABLE 1.—*Results of inoculating cotton bolls with Aspergillus niger*

| Group and kind of material used | Time between inoculation and final examination (days) | Inoculated | | | | Controls | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Injured | | Uninjured | | Injured | | Uninjured | | |
| | | Total number | Number affected | Total number | Number affected | Total number | Number affected | Total number | Number affected | |
| Group 1: Green picked bolls in moist chambers. | 8 | 11 | 3 | 10 | 0 | 10 | 1 | 10 | 0 | Discoloration characteristic only with a few larger lesions. |
| | 9 | 14 | 14 | 13 | 0 | 10 | 0 | .... | .... | One boll decayed completely; three bolls decayed mostly in the interior. |
| | 15 | 57 | 55 | 11 | 1 | 12 | 0 | .... | .... | Most injured inoculations showed decay on the third day, the largest involving three-fourths of the boll and the interior. |
| Total in group | ..... | 82 | 72 | 34 | 1 | 32 | 1 | 10 | 0 | 87.8 per cent of the injured bolls inoculated became affected. |
| Group 2: Growing bolls in the greenhouse | 16 | 4 | 4 | .... | .... | (a) | (a) | (b) | (b) | Typical decay, but not involving more than half the boll; part visible on the third day and the remainder on the eighth day; unaffected portions split and the fungus fruiting appeared on the surface. |
| | 3 | 4 | 4 | .... | .... | | | | | |
| Total in group | ..... | 8 | 8 | .... | .... | .... | .... | .... | .... | 100 per cent infection. |
| Group 3: Growing bolls in the field. | 14 | 25 | 19 | 25 | 0 | 25 | 0 | 25 | 0 | Spots from a fraction of 1 to 2¼ cm. |
| | 13 | 36 | 32 | 5 | 0 | 17 | 0 | .... | .... | The extent of decay varied from a small spot to more than half the boll. |
| | 9 | 13 | 12 | .... | .... | 6 | 0 | .... | .... | |
| Total in group | ..... | 74 | 63 | 30 | 0 | 48 | 0 | 25 | 0 | 85.1 per cent of infection. |
| Total in three groups. | ..... | 164 | 143 | 64 | 1 | 80 | 1 | 35 | 0 | Average infection with wound inoculations, 87.2 per cent. |

[a] No injured checks were used.
[b] A number of uninjured bolls growing at the same time remained healthy.

All of the inoculations included in Table 1 were carried on with cultures of *Aspergillus niger* isolated from decaying cotton bolls. In addition, six growing bolls were inoculated with culture No. 1001, previously isolated by the writer (7) from a potato tuber, in order to ascertain the pathogenicity of this culture to cotton and the character of symptoms produced. The inoculations were made with

one of the series included in Group 3. All of the six bolls became infected and the symptoms were identical with those produced by the strains isolated from cotton. The decay developed to the extent of one-half of the boll, the fungus fruiting on the diseased part. The unaffected portions split open in a typical manner.

It has been noted that under natural conditions in the field earlier sets of the bolls show the largest percentage of infection. Inasmuch as the beginning of the decay is often associated with bollworm injuries, the heavier infection in the earlier bolls may readily be explained by the fact that the bollworm, which is subject to many natural enemies, diminishes in numbers as the season progresses.

## RHIZOPUS NIGRICANS

Unlike *Aspergillus niger*, *Rhizopus nigricans* [4] is a well-known parasite of many cultivated crops (*4*), though the cotton plant has not been on the list of its hosts until comparatively recently. Its association with the sore-shin lesions in Egypt was suspected by Balls (*1, p. 19*) to be more than accidental, although in rather extensive inoculation experiments by Briton-Jones (*3, p. 6–18*) positive results on young seedlings were obtained only with *Rhizoctonia solani*, whereas *Rhizopus nigricans* failed to cause any damage. The same author (*2*), however, definitely established the fact that the latter fungus causes a decay of bolls in Egypt, gaining entrance through insect wounds. A few years later Kirkpatrick (*5*) published a detailed account of his studies of this decay, showing the nature of the damage caused and the resulting losses to the growers.

In the United States this disease may be found in the same localities of the Southwest in which the Aspergillus rot occurs. However, during the seasons of 1925 and 1926 the former appeared to be less prevalent than the latter, and could be found in greater abundance in the San Joaquin Valley than in sections farther south.

As far as cotton bolls are concerned *Rhizopus nigricans*, like *Aspergillus niger*, appears to be principally, if not exclusively, a wound parasite. In most cases it was found to be associated clearly with the circular holes made by the bollworm. Kirkpatrick (*5*) from his studies of this disease in Egypt arrived at the definite conclusion that *R. nigricans* is strictly a wound parasite, and he described several insect agencies responsible for bringing about the infection.

The Rhizopus decay, as it occurs in the Southwest, is quite distinct through all its stages of development. It lacks entirely the pink discoloration characteristic of the Aspergillus decay. The affected portions of the capsule are olive green in color and retain this uniform discoloration until the decayed parts dry up, at which time they become darker. The colored plate accompanying the Briton-Jones paper shows a very dark-brown color of the affected portions of the capsule, which may be observed in this country only at the time when the bolls begin to dry up.

In the earlier stages of the disease the specimens observed by the writer in the United States showed merely a dark-green color of the lesions (pl. 1, C), and little, if any, of the brown or reddish shades. The discoloration of the interior tissues of a freshly decaying boll

[4] L. L. Harter, of the Office of Vegetable and Forage Diseases, Bureau of Plant Industry, examined the writer's culture and confirmed its identification.

is not so uniform and homogeneous and varies from steel gray to slightly purple, with shades of pink and yellow, the latter shades occurring particularly in the seeds (pl. 1, D).

The fruiting stage of the fungus can also be readily distinguished from that of *Aspergillus niger*. The spore masses are not quite so dense as those of the latter organism, and they form a dark-gray or blue-gray rather than a sooty-black powdery film over the boll (compare pl. 1, A, with pl. 1, C, and pl. 2, B). The progress of the decay is somewhat more rapid than that observed in the case of Aspergillus, as for example under humid greenhouse conditions.

With respect to other characteristics, the two diseases are very similar. The Rhizopus decay may break through at points other than the infection wound and may affect either the entire boll or only a part of it. In the latter case the unaffected part may open up and the fungus fructification be seen on the inside, much in the manner shown for the Aspergillus decay. Moreover, it affects not only the capsule but also the immature lint and the seeds, as is clearly evident from the illustrations in Plate 1.

*Rhizopus nigricans* was isolated by the writer also from discolored pedicels, dying small squares, and spots on bracts. It frequently occurs on stem lesions of young cotton seedlings showing symptoms of sore shin, either alone or in association with other fungi. These lesions may be reproduced by means of artificial inoculations with pure cultures of the fungus (*9*), although not so readily as with *Rhizoctonia solani*.

The results obtained by inoculating cotton bolls with *Rhizopus nigricans* are given in Table 2.

TABLE 2.—*Results of inoculating cotton bolls with Rhizopus nigricans*

| Group and kind of material used | Time between inoculation and final examination (days) | Inoculated | | | | Controls | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Injured | | Uninjured | | Injured | | Uninjured | | |
| | | Total number | Number affected | Total number | Number affected | Total number | Number affected | Total number | Number affected | |
| Group 1: Green-picked bolls in moist chambers. | 5 | 12 | 11 | ---- | ---- | 12 | 0 | ---- | ---- | 91.7 per cent infection in Group 1; the outer capsule was destroyed first, then the hard membranes were attacked, but the fiber seemed to remain unaffected. |
| Group 2: Growing bolls in the greenhouse. | 4 | 3 | 3 | ---- | ---- | (a) | (a) | (b) | (b) | Four inoculated bolls decayed completely, the remainder from one-third to one-half. |
| | 3 | 3 | 3 | ---- | ---- | (a) | (a) | (b) | (b) | |
| | 4 | 6 | 6 | ---- | ---- | 3 | 0 | ---- | ---- | Decay affected from one-third to one-half of the boll, breaking out on the side opposite the wound. |
| Total in group | ------- | 12 | 12 | ---- | ---- | 3 | 0 | ---- | ---- | 100 per cent infection in Group 2. |
| Group 3: Growing bolls in the field. | 13 | 34 | 18 | 5 | 0 | 17 | 0 | ---- | ---- | Typical Rhizopus decay affecting from a portion to entire boll. |
| | 9 | 12 | 7 | ---- | ---- | 6 | 0 | ---- | ---- | |
| Total in group | ------- | 46 | 25 | 5 | 0 | 23 | 0 | ---- | ---- | 54.4 per cent of infection in Group 3. |
| Total in three groups. | ------- | 70 | 48 | 5 | 0 | 38 | 0 | ---- | ---- | Average infection with wound inoculations, 68.6 per cent. |

[a] No injured checks were used in these cases.
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## SUMMARY

Two forms of decay of cotton bolls, frequently referred to as "smut" in their later stages, have been found to occur commonly in southwestern United States.

These diseases are not true smuts and have only a superficial resemblance to the smuts.

One of these forms of decay is caused by *Aspergillus niger* Van Tiegh. and the other by *Rhizopus nigricans* Ehr.

The two diseases may be readily distinguished by the discoloration of the affected tissues as well as by the character of the fruiting stages of the parasites.

Both organisms readily produced rot of artificially wounded and inoculated cotton bolls, but failed to affect uninjured bolls.

The infection in the field apparently depends on injuries caused by various insects, the most noticeable of which are those caused by the bollworm. Control measures, therefore, will have to be directed chiefly against these insect enemies.


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# A STUDY OF LEAD ARSENATE AND LIME SPRAY MIXTURES [1]

By P. A. VAN DER MEULEN, *Agent*, and E. R. VAN LEEUWEN, *Associate Entomologist, Bureau of Entomology, United States Department of Agriculture*

## INTRODUCTION

For several years the addition of slaked lime to lead arsenate sprays, for the purpose of decreasing or preventing injury to stone fruits, such as peach and plum, has been recommended by the United States Department of Agriculture. The chemical reactions which may take place in such a mixture have received the attention of at least two investigators. Robinson (*8*) [2] suggests that the reaction which takes place may be represented by the equation:

$$3PbHAsO_4 + Ca(OH)_2 \rightarrow Pb_3(AsO_4)_2 + CaHAsO_4 + 2H_2O.$$

Campbell (*2*) on the basis of certain "semiquantitative experiments" proposes that "for the purpose of discussion" the reaction may be represented by the equation:

$$5PbHAsO_4 + 3Ca(OH)_2 \rightarrow Pb_4(PbOH)(AsO_4)_3 + Ca_3(AsO_4)_2 + 5H_2O.$$

Experiments carried out in the summer of 1925 indicate that neither of the above reactions represents completely the change which takes place, and it appears desirable, therefore, to give an account of this work.

The first part of the present paper embodies the results obtained in laboratory studies of the chemical reactions which may occur in mixtures of acid lead arsenate and lime. The second part of the paper deals with the effectiveness of the mixture in preventing the arsenical injury which results when lead arsenate is used alone in spraying peach orchards.

## LABORATORY EXPERIMENTS

### EARLIER INVESTIGATIONS

The most comprehensive investigations of the arsenates of lead, carried out in recent years, are those of C. C. McDonnell and C. M. Smith (*5*) and of McDonnell and Graham (*4*). Tartar and Robinson (*10*) and G. E. Smith (*9*) have also made a study of these compounds.

If we confine our attention to the compounds which can be prepared by precipitation from solution, there are six arsenates of lead to be taken into account. Using the nomenclature of McDonnell and Smith (*5*), these are: (1) Monolead ortho arsenate, $PbH_4(AsO_4)_2$; (2) dilead ortho arsenate, $PbHAsO_4$; (3) trilead ortho arsenate $Pb_3(AsO_4)_2$; (4) 4, 1, 3, 1–lead-hydroxy arsenate (hydroxy mimetite),

[1] Received for publication April 15, 1927; issued October, 1927. Contribution No. 27 from the Japanese Beetle Laboratory, Riverton, N. J.

[2] Reference is made by number (italic) to "Literature cited," p. 321.

$Pb_4(PbOH)(AsO_4)_3.H_2O$; (5) 5, 2, 4–lead-hydroxy arsenate, $Pb_5(PbOH)_2(AsO_4)_4$; (6) octo-lead arsenate, $8PbO.As_2O_5.\frac{1}{2}H_2O$.

The first of these is obtained only in strongly acid solutions, and is therefore not of importance in the present discussion.

Four arsenates of calcium are described in the literature. These are: (1) Primary calcium arsenate, $CaH_4(AsO_4)_2$; (2) secondary calcium arsenate, $CaHAsO_4$; (3) tertiary (normal) calcium arsenate, $Ca_3(AsO_4)_2$; (4) basic calcium arsenate, $Ca_5(AsO_4)_3OH$.

The first three of these have been recognized for many years. The existence of the last one seems to have been suspected by several investigators. It has been prepared and studied by Tartar, Wood, and Hiner (*11*). These investigators have obtained evidence that an arsenate of calcium more basic than $Ca_5(AsO_4)_3OH$ is not formed by the action of calcium hydroxide solution on this compound at ordinary temperatures.

McDonnell, Smith, and Coad (*6*) studied the effect of atmospheric carbon dioxide on commercial calcium arsenate during storage. They found that carbonation increased the "water-soluble arsenic oxide," but that when the sample was sufficiently basic, the "soluble arsenic oxide" in the completely carbonated samples exceeded 1 per cent in only one case.

On the basis of the work just discussed, it becomes possible to state what reactions will probably occur in a mixture of acid lead arsenate ($PbHAsO_4$) and a large excess of calcium hydroxide, suspended in water. There will be formed basic calcium arsenate, as a result of the decomposition of the acid lead arsenate. The lead arsenate will probably go through several stages of basicity, using the term in the sense of ratio ($PbO:As_2O_5$), and may eventually be deprived of all arsenic oxide, leaving one of the hydrates of lead oxide as a final product. Assuming, for the moment, that lead hydroxide ($Pb(OH)_2$) is the final product we may represent the change which takes place, when the reaction goes to completion, as:

$$3PbHAsO_4 + 5Ca(OH)_2 \rightarrow Ca_5(AsO_4)_3OH + 3Pb(OH)_2 + 3H_2O.$$

## EXPERIMENTS IN 1925

### Action of Calcium Hydroxide on Acid Lead Arsenate

The lead arsenate used in these experiments was a commercial product which was dried at 110° C. to constant weight. Its composition was found by analysis to be:

| | | Found | Calculated for $PbHAsO_4$ |
|---|---|---|---|
| Lead oxide (PbO) | per cent | 64. 94 | 64. 30 |
| Arsenic oxide ($As_2O_5$) | do | 31. 50 | 33. 11 |
| Water ($H_2O$) (by difference) | do | 3. 56 | 2. 59 |

The lead arsenate used was therefore slightly more basic and contained somewhat more water than corresponds to the formula $PbHAsO_4$.

A saturated solution of lime water was prepared from c. p. calcium hydroxide, and analyzed, for each series of experiments, by titration with N–100 hydrochloric acid solution, using phenolphthalein as indicator.

The experiment was carried out as follows: A portion of lead arsenate was weighed out accurately and placed in a jar provided with a glass top and rubber seal, to which was added a definite quantity of lime water and freshly distilled water to give the desired dilution. The jar was then sealed and placed in a motor-driven shaking machine. After shaking at room temperature (about 25° C.) for the required time, the contents of the jar were allowed to settle (usually over night) and 10 c. c. of the clear liquid was removed for titration with N–100 hydrochloric acid. The calcium hydroxide remaining in the solution was subtracted from the amount originally introduced. It was assumed that this difference gave the amount of the calcium hydroxide which had reacted with the lead arsenate. In all these experiments the effect of atmospheric carbon dioxide was reduced to a minimum by working rapidly and removing the tops of the reaction vessels just enough to permit the withdrawal of the samples.

In the first series of experiments the reacting substances were brought together and the shaking continued for a sufficiently long time to permit the reaction to go to completion. The results are summarized in Table 1.

TABLE 1.—*Action of $Ca(OH)_2$ solution on $PbHAsO_4$, after long-continued shaking, at room temperature (25° C.)*

| Lead arsenate used | $Ca(OH)_2$ added | $Ca(OH)_2$ left | $Ca(OH)_2$ used up | Total volume | Time of shaking | $Ca(OH)_2$ used up | |
|---|---|---|---|---|---|---|---|
| | | | | | | Per gram of lead arsenate | Per gram of $As_2O_5$ |
| *Grams* | *Grams* | *Grams* | *Grams* | *C. c.* | *Hours* | *Grams* | *Grams* |
| 0.50 | 0.3180 | 0.1440 | 0.1740 | 200 | 150 | 0.3480 | 1.105 |
| .75 | .3290 | .0920 | .2370 | 200 | 150 | .3160 | 1.003 |
| 2.00 | 1.4927 | .7945 | .6982 | 1,000 | 290 | .3491 | 1.108 |
| 3.00 | 1.4927 | .5764 | .9163 | 1,000 | 290 | .3054 | .969 |

For the production of $Ca_5(AsO_4)_3OH$, 1 gm. of $As_2O_5$ reacts with 1.072 gm. of $Ca(OH)_2$. The other product of the reaction is one of the hydrates of lead oxide.

In two of the jars there was formed a greenish-yellow, flaky substance. This was separated from the white calcium arsenate by stirring, settling, and decantation. The greenish-yellow material, being much heavier, settled quickly. A quantity sufficient for analysis was collected and dried in the air. A determination of lead oxide in the air-dried sample showed it to contain 96.32 per cent PbO. This result corresponds closely to the hydrate $2PbO.H_2O$ (96.12 per cent PbO) which has been described by Luedeking (*3*). Another hydrate, $3PbO.H_2O$, has been described by Pleiszner (*7*), who obtained it by the action of barium hydroxide on warm solutions of lead salts, or cold solutions of PbO. The optical properties of the greenish-yellow crystals obtained in the writers' experiments do not agree well with those ascribed to the hydrate $2PbO.H_2O$, and the composition does not agree with the hydrate $3PbO.H_2O$.

The results leave no doubt, however, as to the fact that at ordinary temperatures the reaction between lead arsenate and calcium hydroxide, if given sufficient time, results in the complete decomposition of

the lead arsenate, with the formation of basic calcium arsenate and a hydrate of lead oxide.

### Rate of Reaction

To ascertain to what extent this reaction proceeds in the ordinary spraying operation a second series of experiments was undertaken, in which weighed quantities of lead arsenate were shaken with known quantities of calcium hydroxide for periods of time which were insufficient for the completion of the reaction. Preliminary experiments having shown that the reaction takes place rather slowly, analysis of the solution was made at the end of 21 and of 37 hours of shaking. The rate of disappearance of calcium hydroxide from the solution may be taken as a measure of the rate of the reaction. The results are given in Table 2.

Table 2.—*Effect of shaking 1 gram of lead arsenate with 200 c. c. of a solution of calcium hydroxide (temperature about 25° C.)*

| Quantities of $Ca(OH)_2$ (in milligrams)— | | | | |
|---|---|---|---|---|
| Originally present | Used up in 0 to 21 hour period | Used up per hour in 0 to 21 hour period | Used up in 21 to 37 hour period | Used up per hour in 21 to 37 hour period |
| 110.9 | 29.4 | 1.40 | 32.5 | 2.03 |
| 126.7 | 30.2 | 1.44 | 28.5 | 1.78 |
| 129.8 | 31.0 | 1.47 | 24.5 | 1.53 |
| 133.0 | 30.2 | 1.44 | 24.1 | 1.50 |
| 136.0 | 28.8 | 1.37 | 25.1 | 1.57 |
| 142.3 | 27.8 | 1.32 | 23.0 | 1.44 |

It is evident that the rate of disapperance of calcium hydroxide does not vary greatly with time, and that it is exceedingly slow. The complete decompositiom of 1 gm. of the lead arsenate used in these experiments requires about 338 mgm. of calcium hydroxide. The average hourly rate of 1.52 mgm. corresponds to the complete decomposition of less than one-half of 1 per cent of the lead arsenate.

On the basis of these results it seems reasonable to conclude that the spray which reaches the foliage consists essentially of unchanged lead arsenate particles, possibly altered superficially to basic calcium arsenate and either lead arsenate or lead hydroxide, and this is mixed with a considerable amount of slaked lime. After such a mixture is exposed to the air, carbonation takes place, so that a deposit of the composition mentioned will soon be altered completely to a mixture of calcium carbonate, acid lead arsenate, and a small amount of basic lead arsenate, or calcium arsenate.

### Rate of Liberation of "Soluble Arsenic"

In a final series of experiments the question of whether the action of atmospheric carbon dioxide results in any large increase in "soluble arsenic" was taken up. The lead arsenate used in these experiments was somewhat basic to begin with, and had a low content of "soluble arsenic." If the chemical action of the lime, followed by atmospheric action, tended to increase the content of "soluble arsenic" appreciably, this should be readily apparent.

In these experiments 2 gm. of lead arsenate were accurately weighed, mixed into a paste with water, and then the cooled milk of lime resulting from the action of about 40 c. c. of water on 6 gm. of quicklime was added. The whole was intimately mixed on a watch glass and allowed to evaporate to dryness in the air. As soon as the material was dry it was powdered in a mortar and then returned to the watch glass, where it was exposed to the air of the laboratory. A number of such samples were prepared and a determination of "soluble arsenic" by the usual method (*1, p. 50*) was made at intervals of several days. The results are as follows:

| Sample No. | Time of exposure (days) | Per cent of soluble arsenic, expressed as $As_2O_5$ |
|---|---|---|
| 1 | 1 | 0.27 |
| 2 | 3 | .44 |
| 3 | 5 | .60 |
| 4 | 8 | .60 |
| 5 | 9 | .68 |
| 6 | 16 | .60 |
| 7 | 23 | .64 |
| 8 | 154 | .65 |
| Lead arsenate without lime | | .44 |

The solutions obtained by treating the above samples with water were tested with phenolphthalein to determine whether the lime had been completely carbonated. All samples gave an alkaline reaction, even the one which had been exposed to the air for 154 days.

In order to determine what the result would have been, had complete carbonation taken place, two additional experiments were carried out. In one of these the water suspension obtained from sample No. 8 was rendered just acid to phenolphthalein by bubbling carbon dioxide through it. The "soluble arsenic" was then determined in the usual manner. Since it was very difficult to avoid a slight excess of the gas, the second experiment consisted in adding 11 gms. of c. p. calcium carbonate powder to 2 gms. of the lead arsenate and 1 liter of carbon-dioxide-free water. The "soluble arsenic" was then determined as before, the results being as follows:

| | Per cent soluble arsenic expressed as $As_2O_5$ |
|---|---|
| Sample 8, acidified with $CO_2$ gas | 4.15 |
| Lead arsenate treated with c. p. $CaCO_3$ | 3.15 |

The results show that, under the conditions of the experiment, carbonation takes place very slowly, and that as long as there is any unchanged calcium hydroxide the amount of "soluble arsenic" is very low. As soon as all the lime has been converted to carbonate, however, there is a large increase in the "soluble arsenic."

## ORCHARD EXPERIMENTS

The results of the laboratory work just described would seem to indicate that the addition of lime to lead arsenate should be effective in preventing injury to peach foliage for a time at least. During the period from the time the spray application is made until the calcium

oxide, not combined as arsenate, is carbonated, the proportion of the "soluble arsenic" is low, and a corresponding freedom from injury may be expected. To determine this point, and also to obtain data on the efficiency of various spray combinations of acid lead arsenate and slaked lime against the Japanese beetle, a number of experiments were conducted in peach orchards in the vicinity of Riverton, N. J., in 1923, 1924, 1925, and 1926.

All of the orchards used for the experimental work consisted of bearing trees in a healthy condition, with a very good growth of foliage. The sprays were delivered by a power sprayer at a pressure of 200 pounds per square inch. Disc nozzles were used with rods. A thorough application was made. In the experiments reported for 1923, 1924, and 1926 the lead arsenate and lime were used in connection with orchard control measures against the Japanese beetle. The lead arsenate and lime were commercial products and no chemical analyses were made of their composition. In 1925, however, a special study was made of the effect of lime and lead arsenate mixtures on peach trees, and chemical analyses were made of the lime and lead arsenate used during that year.

Observations were made at intervals throughout the summer for injury to the fruit, foliage, and wood. The injury, expressed in percentage, is an estimate of the area injured as compared with the rest of the tree. The characteristics of arsenic injury were definite in every instance in which record was made as to the extent of injury.

The experiments made and the results obtained each year are given below.

### EXPERIMENTS PERFORMED IN 1923

The orchard selected for these experiments consisted of the Rochester variety of peaches. Twenty-five trees were selected for each plot and these plots were sprayed June 29. The treatment of the plots was as follows:

Plot 1. Lead arsenate, 3 pounds; water, 50 gallons.
Plot 2. Lead arsenate, 2 pounds; water, 50 gallons.
Plot 3. Lead arsenate, 1 pound; water, 50 gallons.
Plot 4. Lead arsenate, 3 pounds; lime, 3 pounds; water, 50 gallons.
Plot 5. Lead arsenate, 2 pounds; lime, 3 pounds; water, 50 gallons.
Plot 6. Lead arsenate, 1 pound; lime, 3 pounds; water, 50 gallons.
Plot 7. Control, lime, 3 pounds; water, 50 gallons.
Plot 8. Control unsprayed.

#### RESULTS

Observations were made throughout the summer for fruit, wood, and foliage injury, the final results being recorded September 26. At this time heavy deposits of spray residue remained on the fruit and with the exception of plot 1 no foliage, fruit, or wood injury was evident. Plot 1, which had been treated with 3 pounds of lead arsenate to 50 gallons of water, showed foliage injury to the extent of approximately 10 per cent. Aside from this, the trees in all of the plots were vigorous and of good color.

EXPERIMENTS PERFORMED IN 1924

The orchard selected consisted of the following varieties: Hiley Belle, Carman, Old Nixom, and Elberta. The plots consisted of trees of each variety. The treatments were applied June 12, as follows:

Plot 1. Lead arsenate, 1½ pounds; lime, 3 pounds; water, 50 gallons.
Plot 2. Lead arsenate, 1½ pounds; water, 50 gallons.
Plot 3. Control, unsprayed.
Plot 4. Lead arsenate, 2 pounds; lime, 3 pounds; water, 50 gallons.
Plot 5. Lead arsenate, 2 pounds; water, 50 gallons.
Plot 6. Control, unsprayed.
Plot 7. Lead arsenate, 1 pound; lime, 3 pounds; water, 50 gallons.
Plot 8. Lead arsenate, 1 pound; water, 50 gallons.
Plot 9. Control, unsprayed.
Plot 10. Lead arsenate, ½ pound; lime, 3 pounds; water, 50 gallons.
Plot 11. Lead arsenate, ½ pound; water, 50 gallons.

RESULTS

Observations were made at various periods during the season, final results being recorded August 31. Plot 2, treated with lead arsenate 1½ pounds, water 50 gallons, and plot 5 treated with lead arsenate 2 pounds, water 50 gallons, showed foliage injury to the extent of 10 per cent, and slight wood and fruit injury. All other plots were free from arsenical injury. Thus it is shown that the lime added to lead arsenate prevented injury to the foliage in plots 1 and 4.

EXPERIMENTS PERFORMED IN 1925

A 4-year-old peach orchard, planted especially for experimental work by the Japanese Beetle Laboratory, in 1921, consisting of Carman, Hiley Belle, Georgia Bell, and Elberta, was selected. Stone lime [4] from three different manufacturers was used. Three applications of lead arsenate and lime were made May 12, June 12, and July 13, respectively, as follows:

Plot 1. Lead arsenate, 1½ pounds; lime from Bellefonte, Pa., 3 pounds; water, 50 gallons.
Plot 2. Lead arsenate 1½ pounds; lime from Norristown, Pa., 3 pounds; water, 50 gallons.
Plot 3. Lead arsenate, 1½ pounds; lime from Sherwood, Tenn., 3 pounds; water, 50 gallons.
Plot 4. Lead arsenate, 1½ pounds; water, 50 gallons.
Plot 5. Control, unsprayed.

The composition of the lime used in these experiments is as follows: [5]

| | Lime from Bellefonte | Lime from Norristown | Lime from Sherwood |
|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* |
| $SiO_2$ | 0.72 | 0.29 | 0.43 |
| $Al_2O_3$, $Fe_2O_3$ | .61 | .59 | 2.50 |
| CaO | 95.37 | 97.07 | 55.46 |
| MgO | .24 | .26 | 35.18 |
| Loss on ignition | 2.48 | 1.13 | 6.49 |

[4] The authors are indebted to G. J. Fink, chemical director of the National Lime Association, Washington, D. C., through whose courtesy the lime was furnished.
[5] The composition of the lead arsenate used is given on page 2.

### Results

The results from the applications of sprays were very definite. Although a treatment consisting of three applications of lead arsenate at the rate of 1½ pounds to 50 gallons of water is somewhat in excess of the usual recommendations, the writers used this in order to be sure that the effect upon the trees of adding the lime to the lead arsenate would be pronounced. The trees in plot 4, which had been treated with 1½ pounds of lead arsenate to 50 gallons of water, were 90 per cent defoliated August 31, when the final results were recorded. The wood on these trees was so severely injured that only 2 trees of the 24 in the plot were living in 1926. The foliage in plot 3 was injured 5 per cent. All other plots were free from arsenical injury. Apparently a lime high in magnesia is as satisfactory for this purpose as one low in magnesia.

### EXPERIMENTS PERFORMED IN 1926

The same orchard that was used in 1924 was again used in 1926 for conducting these studies. Four plots were used to determine the effect on the foliage of adding lime to lead arsenate, and the treatment was as follows:

Plot 1. Lead arsenate, 1½ pounds; lime, 3 pounds; water, 50 gallons.
Plot 2. Lead arsenate, 1½ pounds; lime, 6 pounds; water, 50 gallons.
Plot 3. Lead arsenate, 1½ pounds; water, 50 gallons.
Plot 4. Control, unsprayed.

### Results

In plot 3, where lead arsenate had been applied without the lime, 10 per cent injury resulted to the foliage, whereas no foliage injury resulted in the plots treated with the lime and the lead arsenate. The results are similar to the results from experiments conducted during previous years, as described above.

The influence of the weather conditions on the physical and chemical action of the lime and lead arsenate mixture is not considered here, but will be discussed in another paper.

## SUMMARY

The action of a solution of calcium hydroxide on acid lead arsenate has been studied in the laboratory.

Under the conditions of the experiments, long-continued action of an excess of calcium hydroxide on acid lead arsenate resulted in decomposition of the lead arsenate, with the formation of basic calcium arsenate and a hydrate of lead oxide.

The reaction takes place slowly, and, under the conditions usually met with in spraying operations, probably less than 1 per cent of the acid lead arsenate is decomposed.

The action of carbon dioxide of the air on the mixture of calcium hydroxide and lead arsenate does not produce an appreciable increase in "soluble arsenic" until after all the lime has been carbonated. Thereafter the amount of "soluble arsenic" increases very materially. In the particular case studied, the increase was from 0.65 per cent to 3.15 per cent of $As_2O_5$.

Orchard experiments with lead arsenate, with and without the addition of slaked lime, have been conducted during the seasons of 1923 to 1926, inclusive, at Riverton, N. J.

It is definitely shown that slaked lime added to lead arsenate prevents peach foliage injury by "soluble arsenic." In one series of experiments, in which a lime high in magnesia was used, no injury resulted.

There is a practical advantage in combining 3 pounds of slaked lime with 1½ pounds of acid lead arsenate in 50 gallons of water.

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# THE PINE TIP MOTH IN THE NEBRASKA NATIONAL FOREST[1]

By SAMUEL A. GRAHAM, *Agent*, and LYNN G. BAUMHOFER, *Junior Entomologist, Forest Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

## INTRODUCTION

The old adage to the effect that hindsight is clearer than foresight applies in forestry as in every other line. If this were not true, the pine tip moth would probably not now be engaging our attention as a pest in the Nebraska National Forest. If the pioneer workers had seen as clearly into the future as we see into the past, forest-pulled planting stock would never have been collected in distant localities for planting in Nebraska. All the trees used in establishing forest growth in the treeless sand-hill region would have been grown from seed, the introduction of the pine tip moth, *Rhyacionia* (*Evetria*) *frustrana* (Comstock), var. *bushnelli* Busck, into the Bessey plantations would probably have been avoided, and one of the most serious problems in connection with the growing of trees in the Nebraska National Forest might never have arisen.

Perhaps this is too strong a statement in view of the fact that there is no definite record of exactly when and in what manner the tip moth reached the Nebraska plantations, but the weight of evidence points strongly toward the probability of its introduction with planting stock shipped in from other localities. Several facts support this assumption. It is known that this insect never voluntarily takes wing even during moderately strong winds and that when it does fly it stays close to the ground, where it can quickly reach shelter. Inasmuch as this habit reduces to a minimum the chance that the moth might have been blown into the plantations by high winds, it is unlikely that the moth could have traveled successfully the distance of 50 or 60 miles from the native pine. On the other hand, it is known that forest-pulled jack pine from Minnesota and possibly from other Lake States, and western yellow pine from the Black Hills, where the tip moth is a common but not a serious pest, were used in some of the earlier plantings in Nebraska. The assumption that the insect was introduced with some of these trees, either as eggs, larvae, or pupae, therefore appears to be justified.

The tip moth appeared in the plantations at Halsey, Nebr., in sufficient numbers to attract attention in the year 1909. Since that time it has found its way into practically all the privately owned plantations in the Kinkaid district. Presumably the insect has been distributed to these plantations in nursery stock from the Bessey nursery, at Halsey.

Each year since 1909 the moth has increased in importance in the plantations. It is responsible for killing some trees by repeated injury year after year, and has retarded growth in the other trees.

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Some trees are barely holding their own against the attack of the pest, while others have been able to make some growth in spite of it.

This is quite a different situation from that existing in most other localities where the tip moth is found. Usually, except in the South where several generations probably occur, the insect is of little economic consequence. Occasionally, however, it becomes epidemic, as in the instance on Nantucket Island reported by Scudder in 1876.[2] From his description it may be concluded that the pest was exceedingly abundant at that time. He states that nearly every new shoot on the infested trees examined had been mined and killed. Since 1876 local outbreaks of a sporadic nature have occurred from time to time in the New England and Atlantic States, but none have been so extensive or serious as the one first described. In fact, aside from this one instance, the pest has attracted little attention. During 1925, however, the moth became abundant on Cape Cod, and another outbreak comparable in proportion and severity to the Nantucket epidemic is now under way.

The tip moth is present generally throughout the Lake States and the Black Hills, but it has never become sufficiently numerous to attract much attention. Its favorite host in the Lake States is jack pine, *Pinus banksiana* (*divaricata*), and in the Black Hills western yellow pine, *Pinus ponderosa*. Throughout the South and West the pest is also widely distributed and is injurious to young hard pines in the large seedling and small sapling stages, but seldom seriously injures trees beyond the sapling stage.

Throughout its range the pine tip moth is characterized by its sporadic outbreaks. One year it will be abundant in certain groves, and perhaps the next year these groves will be almost free from attack, while others in the same general locality will be infested. Even the historic Nantucket outbreak was more or less sporadic in character, inasmuch as the insect was unusually abundant for only a few years and then returned to normal numbers. The factors involved in bringing about these fluctuations are not known. Perhaps the fluctuations are due to variations in the weather, or to fluctuations in the number of the natural enemies of the tip moth, or more probably to a combination of these and other factors.

Why this insect, which normally occurs in such small numbers as to be comparatively unimportant, should be injurious year after year in Nebraska is not clear. Presumably it is due to the fact that some of the environmental factors that hold the pest in check in the native pine forests are lacking in the Nebraska environment. But what these factors are and how they affect the insect are not fully known. It is hoped that further investigations may throw more light on these phases of the problem and that these investigations may lead to the development of effective and economical control.

## PREVIOUS INVESTIGATIONS

The tip moth has been under intermittent investigation in Nebraska since 1909, when the forest supervisor of the Nebraska National Forest reported its presence to the State entomologist and to the Bureau of Entomology of the United States Department of Agri-

[2] SCUDDER, S. H. THE PINE MOTH OF NANTUCKET—RETINIA FRUSTRANA. 20 p., illus. Boston, 1883. (Mass. Soc. Prom. Agr. Pub.)

culture. In the succeeding years up to 1912 observations on the insect were made by representatives of the State entomologist of Nebraska. Much progress was accomplished in studying the biology of the pest. At the end of the season of 1912 the State discontinued the active prosecution of the project. Unfortunately, the results of this work have never been published.[3] No further work was done on this problem until in 1923 the pest was again called to the attention of entomologists by the United States Forest Service. This time representatives of the Bureau of Entomology, United States Department of Agriculture, undertook the investigation in cooperation with the Forest Service. A preliminary survey of the problem was made in 1924. In April, 1925, intensive work on the problem was instituted. The work of the past two seasons has given a fairly complete knowledge of the life history of this pest, and it is the purpose of this paper to present the life history of the tip moth and the present status of the problem. This paper is the product of the above-mentioned cooperative arrangement with the Forest Service. Further investigations concerning the ecology and control of the tip moth are now under way.

## CHARACTER OF INJURY AND SUSCEPTIBILITY OF SPECIES

The pine tip moth mines and kills the expanding buds and the new growth of almost all of the two or three needled pines. It is obvious that when this type of injury is severe the growth of the trees will be much checked. If heavy injury of this sort occurs year after year on the same trees, the result will be a stooling of the tips, which will produce a dwarfed and deformed tree. This causes, during the large seedling and small sapling period, a very material reduction in the rate of height growth of the forest. Injury of this kind seldom results in the death of a tree, although in some cases, when practically all of the tips have been destroyed year after year, trees occasionally have been killed.

All species of pines in the plantations do not appear to be equally susceptible to this pest. Western yellow pine, *Pinus ponderosa*, is the most severely injured. Norway pine, *Pinus resinosa*, jack pine, *Pinus banksiana* (*divaricata*), Scotch pine, *Pinus sylvestris*, and Austrian pine, *Pinus nigra* (*austriaca*), follow in order of susceptibility, judging from counts made in 1925 and 1926, which are shown in Table 1 and represented graphically in Figure 1.

TABLE 1.—*Damage done by the pine tip moth to different species of pine*

| Species of pine | Number of trees | Percentage of terminal tips injured | | Percentage of lateral tips injured | |
|---|---|---|---|---|---|
| | | 1925 | 1926 | 1925 | 1926 |
| Western yellow | 200 | 65.5 | 97 | 45.5 | 71.0 |
| Norway | 200 | ---------- | 96 | ---------- | 64.4 |
| Jack | 200 | 54.5 | 80 | 8.0 | 18.2 |
| Scotch | 100 | 38.0 | 62 | 9.4 | 15.3 |
| Austrian | 100 | ---------- | 3 | ---------- | 3.8 |

[3] The authors are indebted to Myron H. Swenk, State entomologist of Nebraska, for his courtesy in permitting the examination of the notes in his files concerning this insect. At his request, none of the data contained therein have been used in this paper, but a knowledge of the work that had already been done was of considerable assistance in the early stages of the present investigation.

The data in Table 1 are comparable, inasmuch as the trees are all of a highly susceptible size and age. Each year's counts were made on the same plots. The injury to the tips varied from a hollowing of the buds to a mining of the entire new growth.

If it were not for the fact that from the silvicultural point of view the western yellow pine is the tree best suited for sand-hill planting, a substitution of the apparently more resistant Scotch and Austrian pines might well form the basis for tip-moth control; but because of the desirable characteristics of the yellow pine for this sand-hill region, the preservation of that species is to be desired.

Not only does the amount of tip-moth injury vary with the species of pine, but it also varies with the size of the tree. During the first

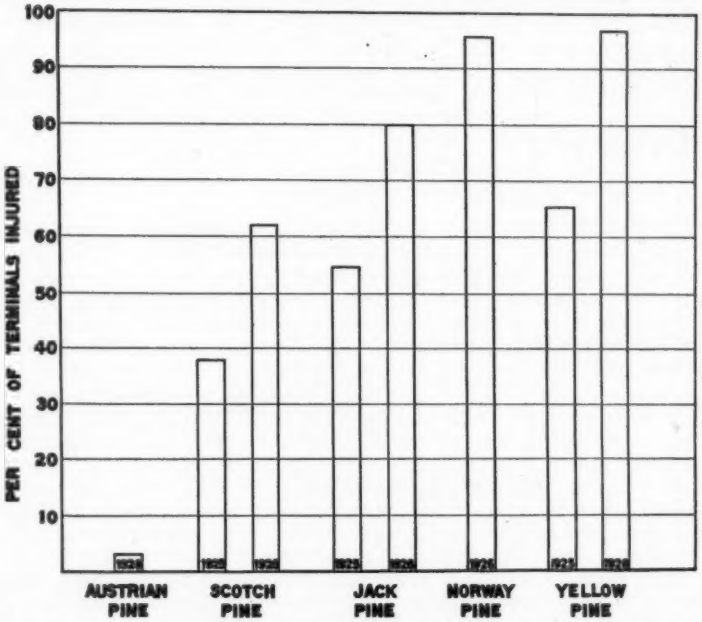


FIG. 1.—Comparison of percentages of terminal tips injured in different species of pine in 1925 and 1926 by *Rhyacionia frustrana bushnelli*

few years after the trees are set out in the plantations very few of them are attacked. This may be because the moths spread slowly from the old plantations into the new ones, or, what seems more probable, because small trees during the first few years are protected more or less by the growth of grass surrounding them. After the trees get above the grass, they become subject to attack and remain highly susceptible until they reach a height of 8 to 9 feet, after which they become less and less susceptible as they increase in height. The taller the trees become, the less likely is the terminal bud of the leading shoot to be destroyed. On the tallest trees in the plantation the greatest injury is done to the lower parts of the trees, whereas the smaller trees are just as likely to have the leading shoot destroyed as to have the laterals attacked.

## SEASONAL HISTORY AND HABITS

Taxonomically the tip moth found in Nebraska is considered to be a geographic variety of the Nantucket pine tip moth, since it does not diverge sufficiently from the eastern form to justify its separation as a distinct species. There are, however, biologic differences, one of which is very striking. The outstanding difference is in the winter habits of the two forms. The eastern variety passes the winter in the pupal stage within the injured pine tips. The Nebraska form, on the other hand, winters as a pupa in the soil beneath the trees. This habit is also characteristic of the tip moth in Minnesota and in the Black Hills.

The moths emerge in early spring, the date of emergence varying from year to year, depending upon the weather. Emergence usually begins early in April and lasts for several weeks. The moths remain active in the plantations until the middle of May or the first of June. They are not rapid fliers and have never been observed flying high above the ground. They spend a large part of their time hidden among the needles of the pine trees, and although they may be very abundant it is unusual to observe many in flight at any one time during the day. About dusk, however, on still, warm evenings a general flight may occur. The moths are seldom observed in voluntary flight in cool or windy weather. So far as known they do not fly at night, or if they do fly at that time, they do not appear to be attracted to light. During the flight period the moths can be shaken from almost any tree; as many as a dozen or more moths can sometimes be shaken from a single small tree. When they are forced to fly under conditions in which they would not voluntarily take wing, they seek the nearest shelter either by flying into a near-by tree or by dropping to the ground. On the ground they often feign death. When at rest on the tree they are very inconspicuous, because their color and shape blend almost perfectly with their surroundings. One of these moths when at rest may easily be mistaken for a bit of dead needle or brown bark. Because of these characteristics they are difficult to observe.

Shortly after the moths emerge oviposition begins. The eggs are about one-half millimeter in diameter, circular in shape, flattened, and light yellow in color. They are usually laid singly, but occasionally in groups of two or three. They are deposited either on the needles, the leaf sheaths, the buds, the bud scales, or the tips; the usual place, however, is on the inner side of a needle. Their size, form, and color would tend to make them inconspicuous, and when it is considered that they are deposited singly it is easy to understand why it is difficult to find them in the field. Oviposition takes place either during the night or early evening. The number of eggs which a moth is capable of laying was not determined in the field. In the laboratory, however, as many as 65 eggs were deposited by a single female. In nature this number might be increased materially. In the laboratory a single moth continued to oviposit throughout a period of one week. The period during which eggs may be found in the field extends from early April into June.

The period of incubation undoubtedly varies considerably, depending upon the weather. During the spring of 1925 the moths had been active and ovipositing for 40 days before the first larva was found in

the field. This would indicate a rather long period of incubation during the cool weather that is characteristic of the spring months. In the laboratory, where the temperature varied from 60° F. at night to slightly above 70° F. during the day, the period of incubation was from 10 days to 2 weeks. The summer brood had a very much shorter period of incubation. At that time the temperature in the laboratory ran between 80° and 90° F. in the daytime and around 70° at night, and the period of incubation varied between 6 and 10 days.

As incubation proceeds the appearance of the eggs changes somewhat. Toward the end of the period the developing embryo may be clearly distinguished through the transparent shell. The eggs are attacked by at least one parasite. The parasitized eggs appear black and opaque instead of yellow. In hatching, the larva breaks through the shell and leaves a rough, irregular opening. In contrast to this an eggshell from which a parasite has emerged can be easily distinguished by the regularly shaped, circular opening through the shell that the adult parasite cuts when it emerges.

When the larvae hatch they are very tiny, about a millimeter and a half in length, with a yellowish-colored body and a black head broader than the body. Their color changes very little throughout the larval period. In the laboratory these young larvae may survive for 24 hours or more without food. Thus, after hatching, a larva has ample time to find a suitable location for its feeding activities. The young larva soon after hatching spins over the spot where it is feeding a thin protective web, which it coats with resin. This web is always located on a new tip, usually at the base of a needle or the base of a bud. Sometimes the larva works on the surface of the stem beneath its web, whereas in other instances the newly hatched larva may tunnel into the base of a needle fascicle and there feed for a short period. Later it burrows either into a bud or into the succulent growth of a new tip. Here it continues to mine until it has completed its development. The web mentioned above may be extended around the base of the bud or along the base of several needle clusters as the larva increases in size. Much of the frass produced during the developmental stage is cast out of the burrow. Thus the opening is kept clear so that the larva can pass in and out freely.

The larvae, so far as observed, always remain either in their tunnels or beneath the protecting web at the opening of the tunnel. When driven from their burrows, however, they may drop by a silken thread. This is particularly true of young larvae. When placed on a new tip or when the web covering is destroyed, the larva very soon spins a new web above itself.

Because of the mechanical difficulties involved in observing individual larvae throughout their developmental period, it was found impossible to observe directly the number of instars through which this insect passes. By measurement of the head capsule of larvae of all sizes collected in the field, however, it was found that they fall into six groups, each of which probably represents an instar. These data, however, were not as conclusive as might be desired because of the intergrading of the groups. There is considerable variation in the size of the head capsule even in the same instar. This is probably due to variations in the abundance or kind of food.

Under the most favorable conditions the length of the larval stage appears to be not less than three weeks. This period may be length-

ened under more adverse conditions. The larvae of the first generation may be found in the field from the middle of May to the first part of July. When fully developed, each larva of the spring generation spins a light silken cocoon inside its burrow and there passes through the pupal stage. On the basis of both laboratory and field observations, the pupal period required two weeks during the season 1925. In 1926, however, in the laboratory 14 to 19 days were required for the pupal period. Pupae of the first generation may be found in the field during the latter two-thirds of June and into July.

Adults of the summer generation begin to emerge during the latter part of June. The period of flight of this generation is comparatively short and lasts less than a month. These moths apparently lay eggs very soon after emergence.

Owing to greater abundance, the summer generation causes much greater injury to the trees than the spring generation. The habits of the larvae, however, are practically the same. They may be observed in the field throughout the greater part of July and August.

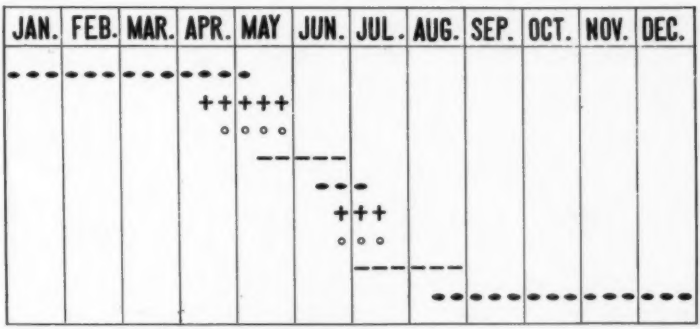


FIG. 2.—Seasonal history of *Rhyacionia frustrana bushnelli* in Nebraska: Solid ovals represent pupal stage; plus signs, adult stage; small circles, egg stage; broken lines, larval stage

Cocooning commences with the oldest larvae about August 10 to 15, and by the end of August all of the larvae have spun cocoons. As has been pointed out before, the cocoons of the second generation are not spun in the tips, as is the case with the spring generation, but the larvae, when full grown, drop to the ground, and in the litter under the trees or just beneath the surface of the sand spin their thin cocoons and transform to pupae. The cocoons spun by the summer generation in the sand seem somewhat heavier than those spun in the tips by the first-brood larvae. This difference, however, may be more apparent than real, because the larvae in spinning the cocoons work in with the threads of silk grains of fine sand. This tends to weight the cocoons and makes them appear heavier than they really are. This mixing of sand with the cocoon makes them much less conspicuous than they would otherwise be, and as a result the cocoons, although abundant, are difficult to find.

Figure 2 illustrates graphically the seasonal history of the tip moth in Nebraska. Figure 3 gives a general idea of the appearance of various stages of the pine tip moth.

In the eastern part of the United States the tip moth also has two generations, but in the Black Hills and in Minnesota only one generation occurs. In both of the latter localities the larvae, after completing their growth, drop to the ground and there spin their cocoons. In the South it is quite possible that more than two generations may occur.

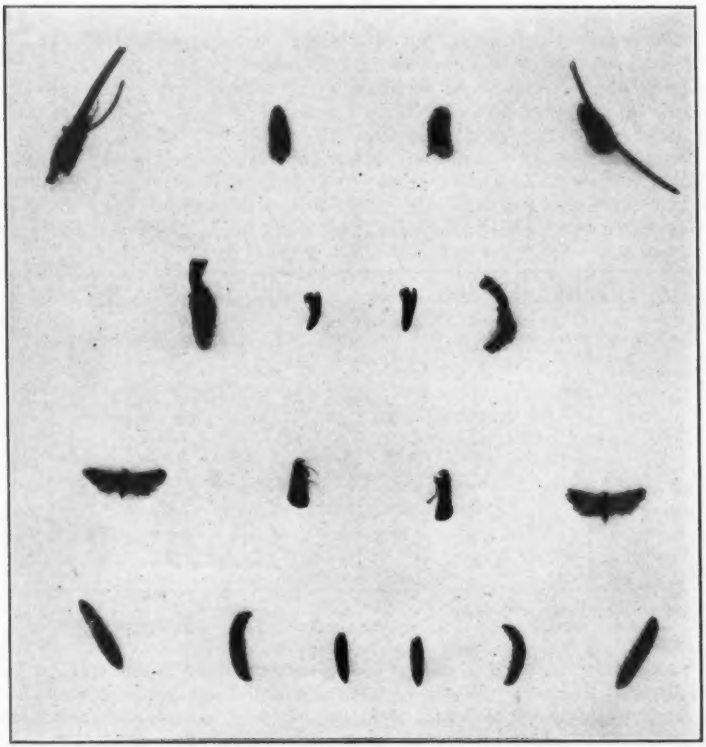

FIG. 3.—*Rhyacionia frustrana bushnelli:* First row (at top), cocoons spun loose in the sand and attached to litter; second row, empty pupal skins, two protruding from cocoons; third row, moths with wings spread and with wings folded; fourth row, four full-grown larvae, and two pupae (in center)

## PARASITES OF THE PINE TIP MOTH NOW PRESENT IN THE PLANTATIONS

When this investigation of the tip moth in the Bessey plantations was first undertaken it was suspected that one of the reasons for the unusual destructiveness of this pest in Nebraska was the absence of its natural enemies in that region. It is possible that this is true and that the most effective enemies are absent, but observations have shown that at least some parasites are working upon this pest in the plantations.

At the time of the introduction of the tip moth a few of its parasites may have been brought in with it, some may have been acci-

dentally blown into the area, and some of the general feeders, native of the region, may have adopted the tip moth as a host. At least 16 species of parasites have been reared from the tip-moth larvae in the Bessey plantations. Of these, 6 are Ichneumonidae, 3 Braconidae, 5 Chalcidoidea, 1 Bethylidae, and 1 Tachinidae. Two chalcidoid species are the most numerous local parasites. One of these is a member of the genus Eurytoma; the other belongs to the genus Haltichella. Next in abundance are two species of the genus Microbracon. In addition to these parasites of the larva, there is at least one undetermined species of egg parasite, a member of the Chalcidoidea. A list of these parasites that have been determined is as follows:

Bethylidae:
*Goniozus longiceps* Kief.
Ichneumonidae:
*Pristomerus*, n. sp.
*Cremastus epagoges* Cush.
*Cremastus* sp.
*Atrometus* sp.
*Epiurus aplopappi* Ashm.
*Itoplectis conquisitor* Say.
Braconidae:
*Microbracon gelechiae* Ashm.
*Microbracon mellitor* Say.
*Microbracon variabilis* Prov.
Chalcidoidea:
*Eurytoma tylodermatis* Ashm.
*Elasmus setosiscutellatus* Cwfd.
*Perilampus chrysopae* Cwfd.
*Habrocytus* sp.
*Haltichella*, n. sp.
Tachinidae:
At least one unidentified species.

In 1925 infested tips of jack pine and western yellow pine were collected and the moths and parasites were allowed to emerge in the laboratory. In 1926 rearings were made from Norway pine and Scotch pine also. Tables 2 and 3 summarize the results of these rearing experiments.

TABLE 2.—*Rearings of tip-moth parasites from local pine tips (spring generation, 1925)*

| Tree species | Number of tips | Number of moths | Number of parasites | Approximate percentage of parasitism |
|---|---|---|---|---|
| Western yellow pine | 600 | 542 | 68 | 11 |
| Jack pine | 200 | 110 | 66 | 38 |

From Table 2 it will be seen that the average percentage of parasitism based on the total rearings from yellow pine in 1925 was 11 per cent, while in jack pine it amounted to 38 per cent.

TABLE 3.—*Rearings of tip-moth parasites from local pine tips (spring generation, 1926)*

| Tree species | Number of tips | Number of moths | Number of parasites | Approximate percentage of parasitism |
|---|---|---|---|---|
| Western yellow pine | 3,500 | 3,147 | 346 | 10 |
| Jack pine | 800 | 168 | 55 | 25 |
| Norway pine | 100 | 48 | 13 | 21 |
| Scotch pine | 600 | 161 | 72 | 31 |

Table 3 gives a similar comparison of the 1926 rearing of spring-generation parasites. It will be noted that the percentage of parasitism was lower than in 1925 but that the excess in jack pine over that in western yellow pine remains in approximately the same ratio. Scotch pine has an even higher percentage of parasitized larvae. It is possible that the higher percentage of parasitization in jack and Scotch pine may explain in part the relative resistance of these trees to tip-moth injury. The reason for the difference in percentage of parasitism in jack, Scotch, and yellow pines is not entirely clear, but it is possible that the explanation may be found in the comparative size of the tips of these trees. The slender twigs and small buds of the jack and Scotch pines provide less effective shelter for the tip-moth larvae than the heavy, large-budded yellow pine, in which they may be burrowed so deeply that the small parasites can not reach them. This does not, however, account for the comparatively high percentage of parasitized larvae in Norway pine, inasmuch as this species has fairly heavy tips. It should be noted, however, in this connection that the data for Norway pine are based upon records from only 100 tips, whereas 600 tips was the smallest number of other species used. Thus the probable observational error in the case of Norway pine is somewhat higher than with the other species. A larger series of observations might change the percentage of parasitized larvae somewhat.

In addition to the parasitic insects that are present in the plantations, there are other agencies that tend to reduce the tip-moth numbers. One species of Cleridae, *Hydnocera pubescens* Lec., has been reared from tip-moth material, and it is possible that still other predacious beetles prey upon the tip moth. Ants have been observed to pick up the full-grown tip-moth larvae that have dropped to the ground for cocooning and carry them away to their nest, presumably for food. Spiders are also known to feed upon the adult moths and upon larvae that have accidentally fallen into the spider webs.

Arboreal birds are finding their way into the plantations, and some of them are doubtless finding in the tip moth a source of food. In fact, it it is not at all uncommon to find injured tips apparently opened by birds and the larvae gone, although no bird has been observed in the act of opening these tips. Birds may become increasingly important as predators.

Work on this problem has not progressed far enough to warrant the recommendation of control measures. A number of promising leads are being followed, however, and it is hoped that effective and economical means of checking this pest may be developed.

## SUMMARY

The pine tip moth, *Rhyacionia frustrana bushnelli*, is one of the most injurious pests present in the Bessey plantations in Nebraska.

It is apparently a geographic variety of the Nantucket pine tip moth, a well known but seldom injurious insect in the eastern part of the United States and Canada.

In Nebraska the pest has been present in large numbers since 1909 and the outbreak shows no sign of abating.

This pest probably was introduced in forest-pulled stock shipped in during the early years of the plantation.

This insect mines the tips of seedling and sapling pines and causes deformity, loss in height growth, and occasionally death of the trees attacked.

The western form winters as pupae in cocoons spun in the ground, whereas the eastern form winters in the infested tips of the trees.

The tip moth has two generations a year. The first flight of moths occurs in April and May and the second late in June and July. The eggs are laid singly on the trees near new growth and the larvae tunnel the buds and young tips. The pupae of the first generation, unlike those of the second, are found in the infested tips.

The severity of infestation varies with species and size of the pine.

A number of parasites are attacking the tip moth, but they do not appear able to hold it in check. It is possible that the most effective parasitic species are not present.

The parasites appear to be more effective in jack and Scotch pine than in western yellow pine, possibly because the larger buds and tips of the yellow pine provide better protection for the larvae.

No satisfactory control measures for this pest have been developed as yet. Further investigations, however, are under way, and it is hoped that some practical means of control may soon be developed.

# EFFECT OF HYDROGEN-ION CONCENTRATION ON THE ABSORPTION OF PHOSPHORUS AND POTASSIUM BY WHEAT SEEDLINGS [1]

By JEHIEL DAVIDSON

*Associate Chemist, Crop Chemistry Laboratory, Bureau of Chemistry and Soils, United States Department of Agriculture*

## INTRODUCTION

Absorption by plants is controlled by two groups of factors, one affecting the availability of the material to be absorbed and the other the absorbing power of the plants. Although the hydrogen-ion concentration of the medium may affect the solubility of the plant-food constituents, its effect on the absorbing power of the plant only was considered in the investigation here reported. Its effect on growth received no attention.

## METHODS AND MATERIALS

The experiments were conducted in the laboratory with water cultures of wheat seedlings prepared as previously described (*5*).[2] Purple-straw wheat was germinated on floating perforated aluminum disks and grown, supported by paraffined paper, in glasses of 225 c c. capacity. In order to prevent absorption of plant food from ungerminated seed before the seedlings were transferred to the glasses, care was taken to keep the seeds on the disks from coming in contact with one another and to prevent their dropping into the germinating pans (*3*).

The seedlings were removed from the disks about two days after germination became visible and were grown for a day or two in tap water before being transferred to the experimental solutions, in order to stimulate root development. Thirty seedlings were grown in each glass. The germinating pans and the water cultures were kept in a large pan surrounded by a moat of oil to prevent injury by ants or roaches.

The seedlings were grown in the experimental solutions for from one to two weeks. Although the solutions were not balanced, the plants looked normal and healthy throughout the experiments, as their needs for growth were adequately supplied by the food materials stored in the mother seeds. Thus it was possible to study the influence of the reaction of the medium on absorption of mineral plant food by the seedlings, without the disturbing influence of previous absorption (*7*).

Chemically pure materials were used for the experimental solutions. The concentrations were made relatively high in order to maintain the buffer properties of the solutions, eliminate deficiency in the plant food constituents as a possible limiting factor, and reduce the relative

[1] Received for publication May 12, 1927; issued October, 1927. Presented in abstract at the seventieth meeting of the American Chemical Society, Los Angeles, Calif., August, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 345.

differences in concentration resulting from absorption by the growing plants. The general character of the results was preserved also in solutions of low concentrations, when proper conditions were maintained.

Absorption was measured by analyzing all of the plant material obtained from each culture. The ash was dissolved and divided into two equal parts, one of which was used for the determination of phosphorus and the other for the determination of potassium. Absolute absorption was measured by comparison with control cultures of seedlings grown in distilled water. Most of the experiments were carried out in triplicate. The hydrogen-ion concentrations were determined colorimetrically.

## EFFECT OF INITIAL HYDROGEN-ION CONCENTRATION ON CHANGES IN REACTION PRODUCED BY GROWING SEEDLINGS IN SINGLE-SALT SOLUTIONS

Seedlings were grown in a number of potassium salt solutions and in a sodium chloride solution which contained 750 parts per million of potassium chloride and equivalents of the other salts. The experiment was conducted in two series. In the first the initial hydrogen-ion concentrations ranged from 5.0 to 5.3 and in the other from 6.3 to 6.7. Hydroxides and acids corresponding to the basic and acid radicals of the salts were used to obtain the desired PH values.

TABLE 1.—*Changes in hydrogen-ion concentrations produced in solutions of potassium salts and in a solution of sodium chloride by growing wheat seedlings*

| Salt | PH of solutions having initial PH of 5.0 [a] after— | | | | PH of solutions having initial PH of 6.7 [b] after— | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 day | 2 days | 4 days | 8 days | 1 day | 2 days | 4 days | 8 days |
| Potassium chloride | 3.7 | 3.5 | 3.5 | 3.6 | 3.9 | 3.7 | 3.5 | 3.8 |
| Potassium nitrate | 3.9 | 4.1 | 4.6 | 5.5 | 4.2 | 4.4 | 4.9 | 6.5 |
| Potassium sulphate | 3.5 | 3.5 | 3.4 | 3.4 | 3.7 | 3.5 | 3.5 | 3.7 |
| Potassium phosphate | 3.9 | 3.5 | 3.4 | 3.4 | 6.2 | 5.9 | 5.6 | 5.6 |
| Potassium acetate | 5.0 | 5.0 | 6.9 | 7.3 | 5.8 | 6.0 | 6.4 | 7.1 |
| Sodium chloride | 4.7 | 4.7 | 5.3 | 6.3 | 4.9 | 5.3 | 6.3 | 6.8 |

[a] The initial PH of the sodium chloride solution was 5.3.
[b] The initial PH of the potassium acetate solution was 6.3.

The growing seedlings increased the acidity of all experimental solutions except that of potassium acetate (Table 1). This is in agreement with the results of Breazeale and LeClerc (*1*), who found titrable acidity in potassium sulphate solutions in which wheat seedlings had been grown, and also with those of Hoagland (*7*), who found that solutions of three potassium salts increased in acidity under the influence of growing barley seedlings, attaining final hydrogen-ion concentrations of 3.4 to 4.0.

Preferential absorption thus seems to favor the bases, at least in the early stages of growth. The fact that sodium, which is not an essential element of plant growth, was also at first taken up in excess over the acid radical suggests that preferential absorption for bases is chemical in nature. The subsequent decrease in acidity in the sodium chloride solutions indicates the migration of sodium back to the solutions. According to Wilfarth, Römer, and Wim-

mer (*14*), even essential elements of plant food migrate back to the soil at certain stages of growth.

The potassium nitrate solutions developed less acidity than the other potassium salts because of preferential absorption of the nitrate radical. In the potassium acetate solutions the acid radical was evidently attacked by microorganisms (*5*).

The initial hydrogen-ion concentration affected the results only in the potassium phosphate solutions. The more acid solutions of this salt behaved like those of the other salts, but the less acid solutions developed only slight acidity.

The experiment was therefore repeated, potassium phosphate and potassium sulphate solutions of several initial hydrogen-ion concentrations being used. The solutions were twice as concentrated as those used before.

TABLE 2.—*Changes in hydrogen-ion concentration produced by growing wheat seedlings in potassium phosphate and potassium sulphate solutions of different initial hydrogen-ion concentrations*

| Initial PH | PH of potassium phosphate solutions after— | | | | PH of potassium sulphate solutions after— | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 day | 2 days | 3 days | 4 days | 1 day | 2 days | 3 days | 4 days |
| 3.6 | 3.3 | 3.3 | 3.3 | 3.3 | 3.4 | 3.4 | 3.3 | 3.3 |
| 4.0 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 |
| 5.0 | 3.8 | 3.7 | 3.7 | 3.6 | 3.7 | 3.6 | 3.6 | 3.6 |
| 6.0 | 5.7 | 5.7 | 5.6 | 5.6 | 3.7 | 3.6 | 3.6 | 3.6 |
| 7.0 | 6.8 | 6.8 | 6.8 | 6.8 | 3.7 | 3.7 | 3.6 | 3.6 |

The results (Table 2) agree with those of the first experiment. All potassium phosphate solutions with initial hydrogen-ion concentrations up to 5.0 behaved like the potassium sulphate solutions. Those with initial PH values of 6.0 and 7.0, however, developed only slight acidity.

The final hydrogen-ion concentrations in these experiments never attained a value lower than 3.3.[3]

## ABSORPTION OF PHOSPHORUS AND POTASSIUM FROM SOLUTIONS OF DIFFERENT HYDROGEN-ION CONCENTRATIONS

The effect of the hydrogen-ion concentration of the medium on the absorption of phosphorous and potassium by seedlings was next studied.

Wheat seedlings were grown for two weeks in solutions containing 1,500 parts per million of monopotassium phosphate which had an original hydrogen-ion concentration of 4.9. The same concentration was used in all subsequent experiments unless otherwise stated. The higher and lower PH values were obtained by using potassium hydroxide and phosphoric acid, respectively.

Normal potassium hydroxide solution was added at the rate of 2.8 and 6.6 c. c. per liter of solution to obtain PH 6.0 and 7.0. The quantity of phosphoric acid required to produce the lower values was relatively insignificant.

[3] On the specific acidity scale, in which PH 7=1, PH 3.3 is 5,000.

The seedlings grown in the solutions which increased markedly in acidity absorbed more phosphorus and less potassium than those grown in the solutions which changed but slightly in hydrogen-ion concentration (Table 3). This is just the reverse of what would be expected in view of the fact that the changes in hydrogen-ion concentration were presumably due to preferential absorption. This seeming abnormality, however, is readily explained by a closer study of the results. The seedlings absorbed relatively more potassium than phosphorus from all solutions, regardless of the initial or final hydrogen-ion concentration (columns 8 and 9, Table 3). This preferential absorption of potassium was responsible for the increased acidity in the solutions with the initial PH 5.0 and lower, as they contained only monopotassium phosphate, which has but slight buffer properties. The solutions with the higher initial PH values, containing mixtures of mono- and di-potassium phosphate, had much higher buffer properties and therefore changed but slightly in hydrogen-ion concentration.

TABLE 3.—*Absorption of phosphorus and potassium by wheat seedlings from potassium phosphate solutions of different initial hydrogen-ion concentrations*

| PH | | Total quantity | | Quantity absorbed | | Ratio of potassium to phosphorus absorbed | Ratio of potassium to phosphorus in solution | Phosphorus (P) equivalent of absorbed potassium | Excess of phosphorus (P) in solution | Excess of phosphorus in solution in terms of $H_3PO_4$ | $H_3PO_4$ required to produce final PH value in 220 cc. of solution | $H_3PO_4$ in excess over that required to produce final PH value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial | Final | $P_2O_5$ [a] | $K_2O$ [a] | $P_2O_5$ | $K_2O$ | | | | | | | |
| | | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | | | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| 3.6 | 3.3 | 19.3 | 14.2 | 14.9 | 11.8 | 151:100 | 126:100 | 7.8 | 1.3 | 4.1 | 1.8 | 2.3 |
| 4.0 | 3.4 | 18.0 | 14.2 | 13.6 | 11.8 | 166:100 | 126:100 | 7.8 | 1.9 | 6.0 | 2.2 | 3.8 |
| 5.0 | 3.6 | 17.8 | 13.9 | 13.4 | 11.5 | 161:100 | 126:100 | 7.5 | 1.6 | 5.1 | 1.7 | 3.4 |
| 6.0 | 5.6 | 13.4 | 16.3 | 9.0 | 13.9 | 295:100 | [b]158:100 | 7.3 | 3.4 | 10.8 | 0.01 | 10.8 |
| 7.0 | 6.8 | 12.6 | 16.0 | 8.2 | 13.6 | 314:100 | [b]201:100 | 5.6 | 2.0 | 6.3 | 0.0004 | 6.3 |
| Controls | ------ | 4.4 | 2.4 | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ |

[a] In 15 seedlings.
[b] Including the potassium added as potassium hydroxide to obtain the required initial PH values.

The excess of phosphoric acid over potassium in the residual solutions does not agree with their final reactions (columns 9 to 13, Table 3). This discrepancy in the solutions with the initial PH 6.0 and 7.0 may be accounted for by their high buffer properties, but in the more acid solutions it can not be explained so easily. It is possible that the dissociation of the free phosphoric acid is decreased by the presence in minute quantities of organic matter, or that the acid is neutralized by small quantities of organic and inorganic bases excreted by the plant roots. In either case the excretions would be so slight that they could not be detected by analysis in aliquots of 15 seedlings.

The experiment was repeated in two series. In one the desired hydrogen-ion concentrations were obtained by the addition of potassium hydroxide and phosphoric acid, in the other by adding hydrochloric acid and sodium hydroxide. The seedlings were grown in the solutions for seven days. The results are given in Table 4.

TABLE 4.—*Phosphorus and potassium absorbed by wheat seedlings from potassium phosphate solutions of different initial hydrogen-ion concentrations obtained with varied reagents*

| Initial PH of solution | From solutions treated with phosphoric acid and potassium hydroxide | | From solutions treated with hydrochloric acid and sodium hydroxide | |
|---|---|---|---|---|
| | $P_2O_5$ [a] | $K_2O$ [a] | $P_2O_5$ [a] | $K_2O$ [a] |
| | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| 3.6 | 15.3 | 13.7 | 13.1 | 12.5 |
| 4.0 | 15.7 | 14.8 | 13.9 | 13.2 |
| 5.0 | 14.9 | 14.2 | 14.3 | 13.2 |
| 6.0 | 12.1 | 15.4 | 11.9 | 15.0 |
| 7.0 | 10.9 | 13.9 | 11.7 | 13.4 |

[a] In 15 seedlings.

The analytical results of the two experiments (Tables 3 and 4) are not comparable, because of the difference in duration and environmental factors, which are never identical in experiments conducted at different times.

The use of sodium hydroxide and hydrochloric acid instead of potassium hydroxide and phosphoric acid had no effect on the results. The influence of the initial PH of the solutions on the absorption of potassium, shown in the preceding experiment, was not corroborated. In both series, however, the absorption of phosphorus is distinctly higher from the solutions with the initial PH 5.0 and lower from those with the initial PH 6.0 and 7.0. The line between these two sets of solutions is just as sharp as in the preceding experiment.

A similar tendency has been recorded by Hoagland (*6*), who used older plants and a complete nutrient solution. His solutions, however, contained enough calcium to precipitate most of their phosphorus at the lower hydrogen-ion concentrations. Similarly, Němec and Gračanin (*9*), who grew rye seedlings in a mixture of soil and sand with the addition of various phosphate fertilizers, found that more phosphorus was absorbed by the seedlings when the reaction of the medium was PH 5.0 than when it was 6.2 or 7.1, but here also the difference in absorption may have been due to differences in solubility. In the present experiments, however, it is clearly not a question of solubility, as all the potassium phosphates are freely soluble. A definite correlation between the hydrogen-ion concentration of the medium and the absorptive power of the plant is therefore established.

## EFFECT OF DURATION OF CONTACT OF SEEDLINGS WITH SOLUTIONS ON ABSORPTION

Six sets of cultures, which were discontinued at definite intervals, were used. As the initial hydrogen-ion concentration of 5.0 had proved to be the line of demarcation between the two rates of absorption, only two series of solutions were used, one with a PH value of 4.9 which expresses the original hydrogen-ion concentration of the solutions (1,500 parts per million of monopotassium phosphate in laboratory distilled water), and the other with a PH value of 7.0, obtained by adding potassium hydroxide.

The final reaction was practically the same in each set, showing that the change in reaction occurs during the first 24 hours (Table

5). The absorption of phosphorus and potassium increased gradually with the duration of the experiment. Absorption of potassium was greater in the neutral (PH 7.0) series, with one exception. Absorption of phosphorus was greater in every set of the acid (PH 4.9) series.

TABLE 5.—*Effect of duration of contact of wheat seedlings with potassium phosphate solutions of different initial hydrogen-ion concentrations on their absorption of phosphorus and potassium*

| Duration of contact | From initial PH 4.9 solutions | | | From initial PH 7.0 solutions | | |
|---|---|---|---|---|---|---|
| | Final PH | $P_2O_5$ [a] | $K_2O$ [a] | Final PH | $P_2O_5$ [a] | $K_2O$ [a] |
| *Days* | | *Mgm.* | *Mgm.* | | *Mgm.* | *Mgm.* |
| 1 | 3.6 | 6.9 | 7.1 | 6.9 | 6.0 | 7.6 |
| 2 | 3.6 | 9.0 | 9.2 | 6.9 | 8.1 | 11.2 |
| 4 | 3.5 | 12.0 | 11.6 | 6.9 | 9.1 | 11.2 |
| 6 | 3.5 | 13.7 | 11.7 | 6.9 | 11.5 | 14.9 |
| 10 | 3.5 | 15.3 | 15.9 | 6.9 | 11.4 | 16.5 |
| 14 | 3.7 | 18.3 | 17.7 | 6.9 | 15.6 | 19.7 |

[a] In 15 seedlings.

Within the period of these experiments, therefore, the duration of contact of the seedlings with the solutions does not affect the differences in the rate of absorption of phosphorus caused by variations in the hydrogen-ion concentration of the medium.

## EFFECT OF AGE OF SEEDLINGS ON ABSORPTION

Five lots of seeds were placed for germination at intervals of 2 to 3 days. After 5 days in the germinating pans the seedlings were transferred to glasses and grown in tap water until the last lot was ready for use. The seedlings, therefore, differed in their stages of advancement by periods of 2 days and in one case by 3 days. These differences in age were discernible throughout the experiment. All seedlings were grown in the experimental solutions for 6 days.

Both the absorption and final reaction of the solutions were distinctly affected by the age of the seedlings (Table 6). In the acid series the acidity produced by the growing seedlings decreased as their age at the time of transfer to the solutions increased. In the neutral series a similar tendency was presumably obscured by the buffer properties of the solutions. The absorption of potassium and phosphorus decreased also in both series as the initial age of the seedlings increased, the rate of absorption being highest at the lowest starting age. The 5-day seedlings absorbed four times as much potassium and four to five times as much phosphorus as the 14-day seedlings. This impairment of absorbing power with age may be due to the fact that there was no absorption during growth in a medium devoid of mineral plant food. It is also possible that the rate of absorption decreases as the seedlings advance in age, regardless of the presence or absence of plant food in the medium.

TABLE 6.—*Effect of age of wheat seedlings on absorption of phosphorus and potassium from potassium phosphate solutions of different initial hydrogen-ion concentrations*

| Age of seedlings [a] | From initial PH 4.9 solutions | | | From initial PH 7.0 solutions | | |
|---|---|---|---|---|---|---|
| | Final PH | $P_2O_5$ [b] | $K_2O$ [b] | Final PH | $P_2O_5$ [b] | $K_2O$ [b] |
| *Days* | | *Mgm.* | *Mgm.* | | *Mgm.* | *Mgm.* |
| 14 | 4.1 | 8.0 | 6.15 | 7.1 | 7.3 | 5.59 |
| 12 | 4.0 | 9.8 | 8.01 | 7.1 | 7.6 | 7.31 |
| 10 | 3.8 | 11.4 | 9.39 | 7.0 | 9.9 | 10.50 |
| 7 | 3.3 | 14.4 | 14.00 | 6.9 | 10.1 | 13.80 |
| 5 | 3.3 | 21.5 | 17.90 | 6.9 | 14.0 | 16.10 |
| Controls | | 5.0 | 2.28 | | 5.0 | 2.28 |

[a] Count started with day the seed was set to germinate. [b] In 15 seedlings.

The relative absorption of potassium was irregular, whereas that of phosphorus was consistent in both series. The differences in the absorption of phosphorus caused by the hydrogen-ion concentration of the medium, however, decreased as the starting age increased The difference for the starting age of 14 days was about 9 per cent and that for the age of 5 days was about 30 per cent.

Total absorption, relative absorption, and the final reaction of the medium are correlated at least in a general way. It is not unlikely, therefore, that all are conditioned by the same factor.

## EFFECT OF CONCENTRATION ON ABSORPTION

Solutions of relatively high concentrations were used in these experiments for reasons stated at the outset. As normal soil solutions rarely attain such concentrations, however, it was necessary to determine the effect of concentration on the differences in absorption. Accordingly, six sets of cultures were grown for 9 days in potassium phosphate solutions ranging in concentration from 50 to 3,000 parts per million.

As was to be expected, the buffer properties of the solutions in the neutral series decreased gradually in the lower concentrations, beginning with that of 500 parts per million (Table 7). The apparent exception shown by the solution of the lowest concentration was evidently due to the total removal of the potassium phosphate by the plants, PH 5.4 being essentially the hydrogen-ion concentration of distilled water which has come into equilibrium with the carbon dioxide of the air. The final reaction of the corresponding solution in the PH 4.9 series may be similarly explained, the removal of potassium phosphate being not quite complete in this case.

TABLE 7.—*Effect of concentration on absorption of phosphorus and potassium by wheat seedlings from potassium phosphate solutions of different initial hydrogen-ion concentrations*

| Concentration of solutions [a] | From initial PH4.9 solutions | | | From initial PH 7.0 solutions | | |
|---|---|---|---|---|---|---|
| | Final PH | $P_2O_5$ [b] | $K_2O$ [b] | Final PH | $P_2O_5$ [b] | $K_2O$ [b] |
| | | *Mgm.* | *Mgm.* | | *Mgm.* | *Mgm.* |
| 3,000 | 4.0 | 19.8 | 16.6 | 6.9 | 13.5 | 15.6 |
| 1,500 | 3.5 | 18.8 | 15.7 | 6.9 | 11.8 | 14.5 |
| 500 | 3.3 | 10.0 | 9.9 | 5.9 | 10.3 | 15.5 |
| 200 | 3.3 | 7.6 | 7.7 | 3.6 | 8.7 | 11.6 |
| 100 | 3.6 | 7.2 | 5.7 | 3.6 | 8.1 | 9.8 |
| 50 | 4.1 | 6.1 | 4.1 | 5.4 | 6.8 | 5.9 |
| Control | | 4.4 | 2.7 | | 4.4 | 2.7 |

[a] Parts per million. [b] In 15 seedlings.

The relative absorption of phosphorus from the solutions with concentrations of 1,500 and 3,000 parts per million was the same as in the previous experiments, but it deviated markedly from the normal course in the lower concentrations, beginning with that of 500 parts per million. This was probably due to the changes in hydrogen-ion concentration in the solutions of the neutral series, resulting from their decreased buffer properties. The absorption of potassium was higher from all four concentrations below 1,500 parts per million in the neutral series.

The initial hydrogen-ion concentration of the medium had no effect on the relative absorption of phosphorus from concentrations which approach that of the soil solution. However, the soil solution is constantly replenishing its stock of available plant food. Accordingly, the experiment on concentration was repeated, solutions being renewed daily. The seedlings were grown in the solutions for 8 days.

The PH values of the solutions discarded daily show that the power of the seedlings to change the initial hydrogen-ion concentration of the solutions diminished with every daily renewal (Table 8). The main object of renewing the solutions—to prevent large variations from the initial hydrogen-ion concentrations in the neutral series—was thus attained.

TABLE 8.—*Changes in reaction produced by growing wheat seedlings in daily renewed potassium phosphate solutions of different concentrations and different initial hydrogen-ion concentrations*

| Concentration of solution [a] | PH of initial PH 4.9 solutions after— | | | | | | | | PH of initial PH 7.0 solutions after— | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days | 8 days | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days | 8 days |
| 1,500 | 3.6 | 4.1 | 4.7 | 4.7 | 4.8 | 4.8 | 4.8 | 4.8 | 6.9 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| 500 | 3.6 | 3.8 | 4.1 | 4.3 | 4.8 | 4.9 | 4.9 | 5.0 | 6.0 | 6.9 | 6.9 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| 200 | 3.5 | 3.7 | 4.0 | 4.1 | 5.1 | 5.1 | 5.2 | 5.3 | 5.1 | 6.1 | 6.3 | 6.3 | 6.3 | 6.3 | 6.4 | 6.3 |
| 100 | 3.4 | 3.7 | 4.0 | 4.1 | 5.1 | 5.1 | 5.2 | 5.3 | 3.9 | 5.4 | 6.1 | 6.2 | 6.2 | 6.3 | 6.3 | 6.3 |
| 50 | 3.4 | 3.6 | 4.0 | 4.1 | 5.2 | 5.4 | 5.4 | 5.4 | 3.7 | 5.0 | 5.5 | 5.8 | 5.9 | 6.1 | 6.1 | 6.1 |

[a] Parts per million.

Absorption of phosphorus was greater from the acid than from the neutral solutions, irrespective of their concentrations, when through renewal they simulated the soil solution (Table 9). It is probable, therefore, that this correlation of absorption with the hydrogen-ion concentration of the medium properly modified may also apply to natural conditions of plant growth.

TABLE 9.—*Effect of concentration on absorption of phosphorus and potassium by wheat seedlings from potassium phosphate solutions of different initial hydrogen-ion concentrations renewed daily*

| Concentration of solution [a] | From PH 4.9 solutions | | From PH 7.0 solutions | |
|---|---|---|---|---|
| | $P_2O_5$ | $K_2O$ | $P_2O_5$ | $K_2O$ |
| | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| 1,500 | 9.7 | 9.25 | 7.0 | 8.5 |
| 500 | 8.5 | 8.90 | 6.6 | 10.0 |
| 200 | 7.5 | 8.13 | 5.9 | 9.40 |
| 100 | 7.2 | 8.32 | 5.9 | 8.80 |
| 50 | 6.8 | ---- | 5.5 | 7.40 |
| Control | 3.7 | 1.70 | 3.7 | 1.70 |

[a] Parts per million.

## DISTRIBUTION OF ABSORBED CONSTITUENTS AMONG ROOTS, TOPS, AND SEEDS

In ashing the plant material for analysis a blackening, considered to be indicative of high phosphorus content, was observed in the roots of the seedlings grown in the acid solutions. This led to a study of the distribution of the absorbed phosphorus and potassium among tops, roots, and mother seeds. The usual procedure was followed. At the end of the experiment the seedlings were carefully separated into tops, roots, and seeds, and then oven dried, weighed, and ashed. Aliquots of 45 seedlings were used for analysis.

The dry weights of the tops were highest in the acid series and lowest in the controls (Table 10). The dry weights of the roots were highest in the controls, which is in accord with the common observation that a medium poor in plant food may stimulate root development. The weights of the mother seeds were lowest in the controls, but practically identical in the other two series.

TABLE 10.—*Distribution of potassium and phosphorus absorbed by wheat seedlings from potassium phosphate solutions of different initial hydrogen-ion concentrations in tops, roots, and mother seeds*

| Part of plant | From initial PH 4.9 solutions | | | From initial PH 7.0 solutions | | | From controls[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dry weight | $P_2O_5$ | $K_2O$ | Dry weight | $P_2O_5$ | $K_2O$ | Dry weight | $P_2O_5$ | $K_2O$ |
| | *Gm.* | *Mgm.* | *Mgm.* | *Gm.* | *Mgm.* | *Mgm.* | *Gm.* | *Mgm.* | *Mgm.* |
| Tops | 0.50 | 33.4 | 35.4 | 0.44 | 20.1 | 30.6 | 0.38 | 9.2 | 6.6 |
| Roots | .14 | 5.8 | 3.3 | .15 | 6.7 | 7.0 | .17 | 1.7 | .5 |
| Seeds | .20 | 11.3 | 3.5 | .19 | 9.2 | 4.8 | .16 | 2.0 | .3 |
| Total[b] | .84 | 50.5 | 42.2 | 1.78 | 36.0 | 42.4 | 1.73 | 12.9 | 7.4 |

[a] Seedlings grown in distilled water. [b] In 45 seedlings.

The roots in the neutral series had, contrary to expectation, the highest phosphorus content, but they also had a relatively larger potassium content. It would seem, therefore, that blackening of ash is an indication of an excess of phosphorus over bases rather than of high phosphorus content in an absolute sense.

The total potassium content of the seedlings in the two series was about the same, but in the distribution of this element the tops of the acid series were more favored. They also contained the entire excess of phosphorus absorbed by the seedlings of this series. This emphasizes the significance of the hydrogen-ion concentration of the medium as a factor in the physiological availability of phosphorus to plants brought out in these experiments.

## DISCUSSION

The behavior of seedlings with respect to absorption of phosphorus is analogous to that of certain inorganic gels which, according to Starkey and Gordon (*12*), also absorb more phosphorus from potassium phosphate solutions as their hydrogen-ion concentrations increase. Here, however, preferential absorption was always in favor of the potassium cation, regardless of the rate of absorption

of phosphorus. The phenomena observed can be more plausibly explained on the basis of the isoelectric relations of the colloidal components of the living cell, particularly the proteins. Robbins (*11*) offered this explanation to account for the differences in absorption of water and dyes by potato tissue on immersion in solutions of different hydrogen-ion concentrations.

According to Pearsall and Ewing (*10*), the isoelectric point of wheat lies between PH 3.3 and 4.5. The more acid limit happens to be identical with the lowest limits of acidity produced by the wheat seedlings in these experiments. Csonka, Murphy, and Jones (*2*) found that the isoelectric point of proteins lies between higher PH values, approaching more closely the hydrogen-ion concentration the cell sap in 2-week wheat seedlings, which is about PH 6.0, according to Hurd (*8*). However, it is safe to assume that the isoelectric points of proteins of the living cell are not identical with those of isolated proteins which have been subjected to various treatments in the process of extraction and purification. It is further to be assumed that the hydrogen-ion concentration of the cell content is not made identical with that of the medium, but only modified by it. The preferential absorption of potassium is accordingly explainable by the supposition that as long as the acidity of the medium does not increase beyond PH 3.3 most of the colloids in the wheat seedlings are on the electro-negative side of their isoelectric points and therefore combine with basic radicals in excess over acid radicals. However, as the acidity of the solutions increases because of the preferential absorption of potassium, some colloids pass to the electro-positive side of their isoelectric points and an increased absorption of phosphorus follows. When the acidity of the medium increases momentarily beyond PH 3.3 the absorption of phosphorus increases rapidly and this value is restored, the preferential absorption for potassium being thus maintained.

The assumption that there is a relatively wide range in the isoelectric point of the ampholytes of growing plants explains not only the absorption phenomena observed in the experiments here reported but also the general mechanism of absorption of the living cell. This diversity of isoelectric points, allowing the occurrence of both electro-positive and electro-negative ampholytes within certain limits of hydrogen-ion concentration, makes possible the simultaneous absorption of cations and anions.[4]

Although it is realized that it is not always safe to apply to field conditions conclusions drawn from laboratory experiments, the facts brought out in these experiments may have a practical bearing on plant production. For example, the high absorptive power of young seedlings would suggest caution in the practice of thinning, in order not to remove too much available plant food from the immediate vicinity of the remaining plants (*4*). The beneficial effect of transplanting young seedlings may be explained on the same basis. When thick their intensive feeding exhausts the soil, so that they are benefited by being transplanted into fresh soil. They also bring to their permanent ground a store of plant food derived from other sources, which in a way is equivalent to an application of fertilizers.

[4] Stearn's (*13*) hypothesis that a mixture of ampholytes acts as an individual with one isoelectric point lying somewhere between the PH values of the components, would hardly apply to the proteins of living organisms, as these are presumably deposited in separate layers or in different cells and would be expected to react individually.

The fact that the physiological availability of phosphorus to plants depends, among other factors, upon the hydrogen-ion concentration of the medium, emphasizes anew the importance of soil reaction. This may be one of the reasons why some plants appear to prefer an acid medium. It also suggests that the beneficial effect of the application of potassium fertilizers may be due partly to the fact that they increase the acidity in the immediate vicinity of the growing plants. The increased absorption of potassium, then, results indirectly in an increased absorption of phosphorus and perhaps of the other acid-forming elements of plant food also.

## SUMMARY

Wheat seedlings were grown in potassium phosphate solutions of different initial hydrogen-ion concentrations.

Relatively more potassium than phosphorus was absorbed by the seedlings, irrespective of the initial hydrogen-ion concentration of the solution. In the solutions with initial hydrogen-ion concentrations of 5.0 and lower this preferential absorption of potassium resulted in increased acidity. In solutions with initial hydrogen-ion concentrations of 6.0 and 7.0 the increase in acidity was but slight, owing to the buffer properties of the solutions.

More phosphorus was absorbed by the seedlings from the solutions with initial PH values of 5.0 and lower than from those with PH values of 6.0 and 7.0. As all potassium salts of phosphoric acid are soluble, this tends to show that the physiological availability of phosphorus depends upon the hydrogen-ion concentration of the medium.

The general character of the results was not affected by the duration of the experiments nor by the age of the seedlings. Neither was it affected by the concentration of the solution, provided the differences in initial reactions in the solutions of the lower concentrations were maintained by daily renewal.

The excess of phosphorus absorbed from the acid solutions was found in the tops of the seedlings. The tops also had a higher potassium content than those from the neutral solutions. The roots from the neutral solutions contained more phosphorus and almost twice as much potassium as those from the corresponding acid solutions.

The power of the seedlings to absorb phosphorus and potassium decreased as they advanced in age.

The absorption phenomena observed in these experiments, as well as the absorption of cations and anions by living cells in general, are explained by the assumption that there is a relatively wide range in the isoelectric points of individual protoplasmic ampholytes.

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# NOTES ON THE LIFE HISTORY OF THE BUD MOTH, SPILONOTA OCELLANA D. & S.[1]

By S. W. Frost

*Associate Professor of Economic Entomology, The Pennsylvania State College*

## INTRODUCTION

A detailed study of the life history and habits of the eye-spotted bud moth, *Spilonota ocellana* D. & S., was started in 1917 and continued for five years. The investigations were conducted in an outdoor insectary at Arendtsville, Pa., and frequent trips were made to near-by orchards to correlate insectary and orchard conditions. Synonymy, food plants, distribution, and bibliography have purposely been omitted, for these have been adequately discussed by Porter,[2] in his bulletin "The Bud Moth." The present paper contributes life history studies with special reference to conditions in southern Pennsylvania.

## NATURE OF THE INJURY

The injury caused by the larvae of the bud moth in Pennsylvania may be described under the following headings: (1) Early injury to buds, foliage, and developing fruit by the partly grown, overwintering larvae; and (2) late injury to mature fruit by larvae which hibernate the following winter.

The partly grown, overwintering larvae become active early in the spring and burrow into the opening flower buds (fig. 1, D), feeding upon the blossoms and thereby lessening the set of fruit. As the leaves unfold, the larvae tie them together with silk and feed within the clusters thus made. Newly formed apples are frequently inclosed in such clusters (fig. 1, C) and are chewed by the larvae, causing them to drop or to become disfigured when they mature. The fruit stems are sometimes gnawed, which weakens them and causes the small fruit to drop. Often the larvae bore into the terminal shoots and during some years considerable injury results. Sanders and Dustan (1919)[3] report that the injury to blossom clusters in Nova Scotia in 1918 was 35.8 per cent and that in some cases the set of fruit was reduced as much as 79.9 per cent. The size of the fruit from injured clusters was also considerably smaller. Although the percentage of injury in Pennsylvania has not been as serious as this, it is evident that this insect can, at times, cause considerable loss.

Late in the summer the young larvae bite into the fruit, making small scars. Such injury is most common where a leaf is in contact with a fruit or where two fruits hang together. Plate 1, H, shows a mature fruit that has been injured by the feeding of a bud-moth

[1] Received for publication Apr. 27, 1927; issued October, 1927. Published by permission of the director of the Pennsylvania Agricultural Experiment Station as a part of project No. 608. Contribution from the Department of Zoology and Entomology, the Pennsylvania State College, Technical paper No. 427.

[2] Porter, B. A. The bud-moth. U. S. Dept. Agr. Bul. 1273, 20 p., illus. 1924.

[3] Sanders, G. E., and Dustan, A. G. The apple bud-moths and their control in Nova Scotia. Canada Dept. Agr. Ent. Branch Bul. 16: 5–30, illus. 1919.

larva. The discolored area also shows the position of the leaf beneath which the larva was feeding at the time the injury was produced. The most serious injury in Pennsylvania is of this type. J. R. Eyer, formerly of this station, found 14.7 per cent of fruit on sprayed trees injured by bud-moth larvae during 1917. In 1918, 5.1

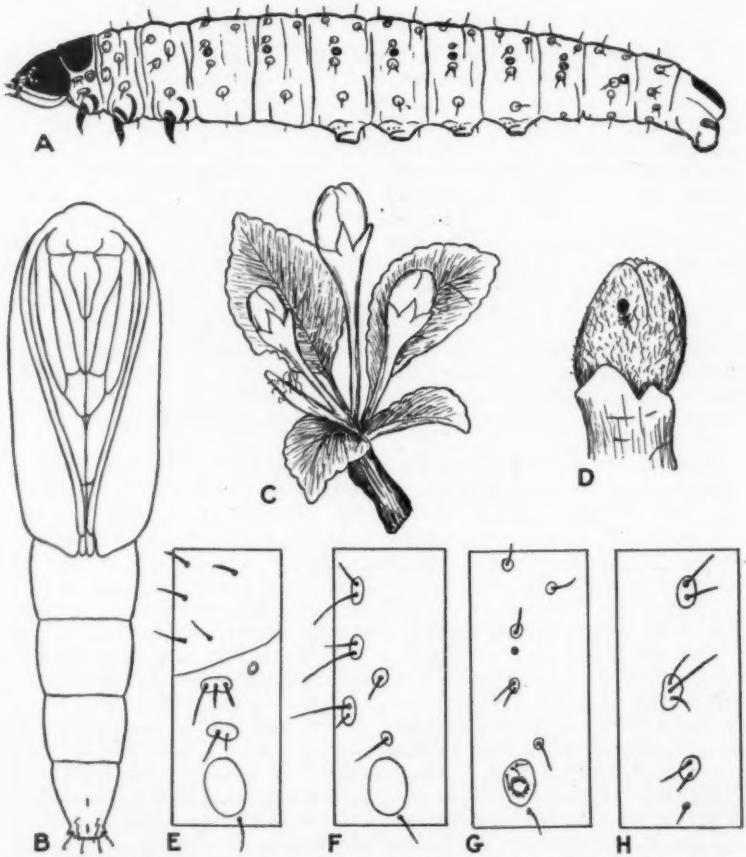
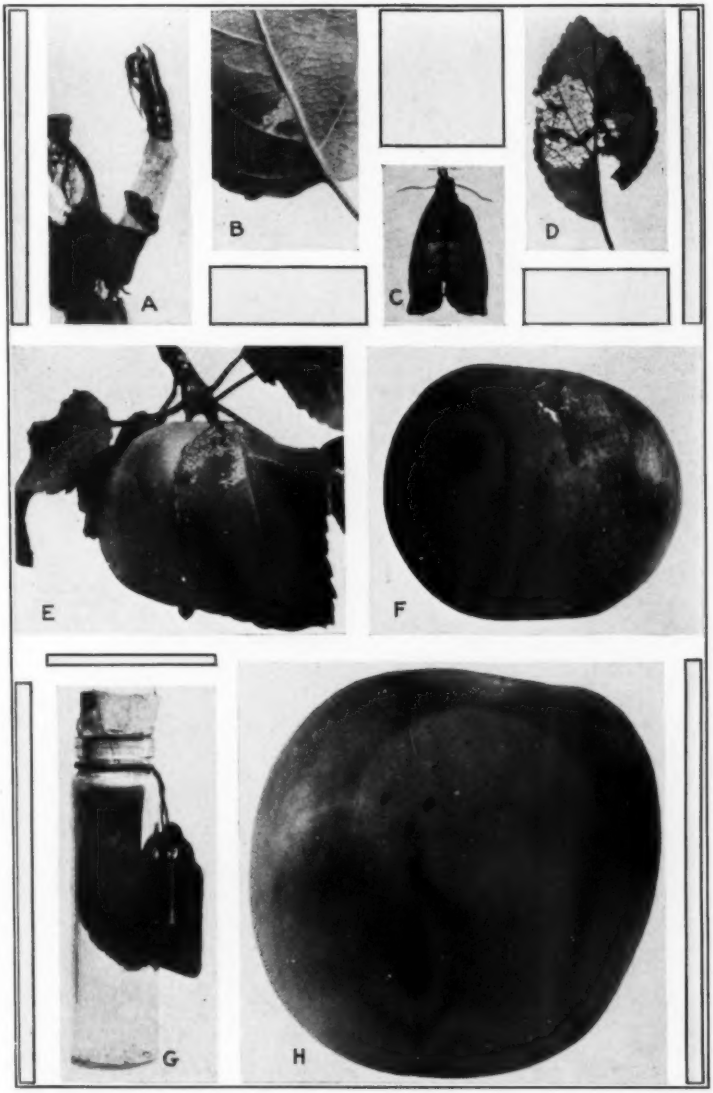



FIG. 1—A, larva of bud moth; B, pupa of bud moth; C, feeding case and leaf cluster; D, bud-moth injury on apple bud; E, prothoracic segment of larva; F, mesothoracic segment of larva; G, third abdominal segment of larva; H, ninth abdominal segment of larva

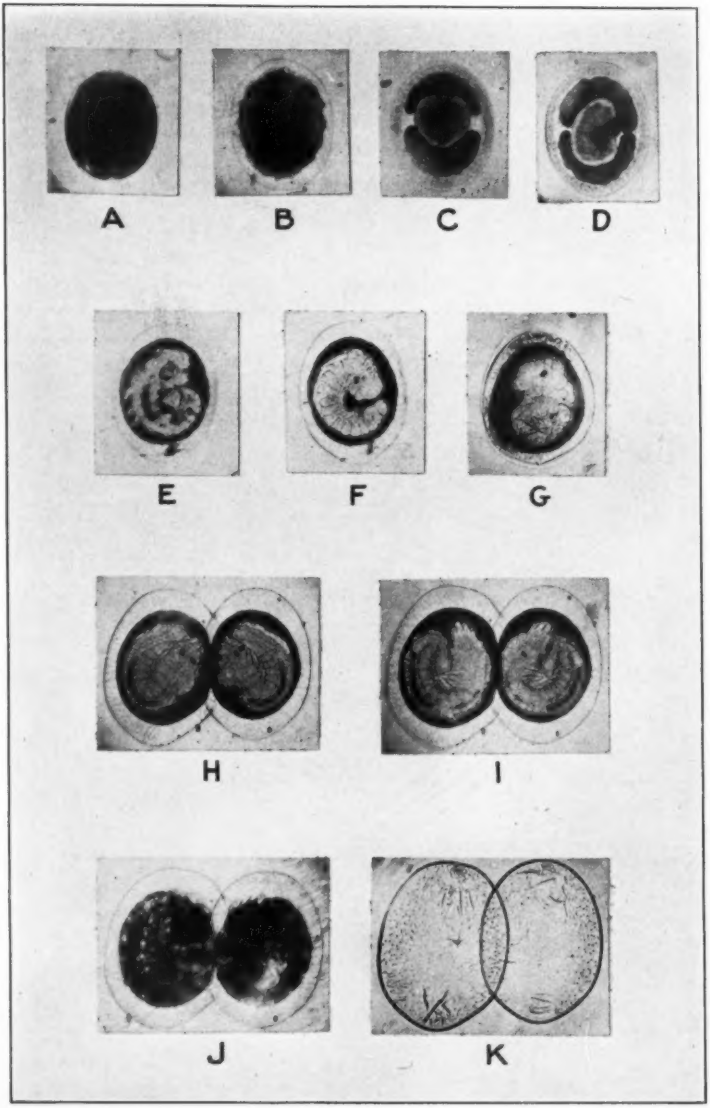
per cent, and in 1919, 10 per cent of the fruit on unsprayed trees were injured by this species.

## THE ADULTS

The adults (pl. 1, C) were rarely observed flying in orchards and records show that they are seldom attracted to lights or sugar baits. In captivity one can rear them with ease if they are provided with water or sugar solution. This seems necessary in order to keep the adults from dying prematurely.




A.—Empty pupal case.
B.—Position of larva immediately after hatching, under silken tent.
C.—Adult of Spilonota ocellana D. & S.
D.—Feeding case and manner of feeding of larva.
E.—Leaf tied to fruit by larva of bud moth.
F.—Same as in E with leaf removed.
G.—Rearing bottle, with bud-moth pupa on leaf, natural size.
H.—Feeding punctures of bud-moth larva, showing discolored area on fruit caused by leaf cutting off the sunlight.




A.—Egg immediately after oviposition.
B.—Egg showing migration of yolk to opposite ends.
C.—Yolk gathered at opposite ends of egg, embryo visible in middle.
D.—Yolk flowing about embryo.
E.—Embryo 54 hours old, showing leg buds.
F.—Embryo showing segmentation and legs pressed against thorax.
G.—Horizontal revolutions of embryo.
H.—Six-day-old embryos after vertical revolution.
I.—Advanced stage of H, showing abdomen elongated and curved at the tip.
J.—Mature embryo, head fully colored, and yolk consumed.
K.—Empty egg shells.

The length of life of the adult moth varies from 5 to 26 days, the average for both males and females being approximately 14 days. A length-of-life record kept in 1919 is shown in Table 1.

TABLE 1.—*Length of life of adult male and female eye-spotted bud moths, 1919*

| Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|
| Number of adults | Adults issued | Adults died | Length of life (days) | Number of adults | Adults issued | Adults died | Length of life (days) |
| 1 | June 2 | June 18 | 16 | 1 | June 2 | June 24 | 22 |
| 1 | June 3 | June 24 | 21 | 1 | June 3 | June 24 | 21 |
| 1 | June 5 | June 23 | 18 | 1 | June 4 | June 12 | 8 |
| 1 | June 6 | June 16 | 10 | 1 | June 5 | June 23 | 18 |
| 1 | June 6 | June 23 | 17 | 1 | June 6 | June 16 | 10 |
| 1 | June 6 | June 24 | 18 | 1 | June 6 | June 19 | 13 |
| 1 | June 6 | July 2 | 26 | 1 | June 6 | June 24 | 18 |
| 1 | June 7 | June 16 | 9 | 1 | June 6 | July 2 | 26 |
| 1 | June 7 | June 17 | 10 | 1 | June 7 | June 16 | 9 |
| 1 | June 7 | June 18 | 11 | 1 | June 7 | June 17 | 10 |
| 1 | June 7 | June 19 | 12 | 1 | June 7 | June 24 | 17 |
| 1 | June 7 | June 20 | 13 | 1 | June 8 | June 23 | 15 |
| 1 | June 7 | June 21 | 14 | 1 | June 9 | June 23 | 14 |
| 1 | June 8 | June 18 | 10 | 1 | June 9 | June 24 | 15 |
| 1 | June 9 | June 16 | 7 | 1 | June 9 | June 28 | 19 |
| | | | | 1 | June 11 | June 26 | 15 |
| | | | | 1 | June 12 | June 25 | 13 |
| | | | | 1 | June 12 | June 28 | 16 |
| | | | | 1 | June 13 | July 2 | 19 |
| | | | | 1 | June 15 | June 20 | 5 |
| | | | | 1 | June 15 | June 25 | 10 |

NOTE.—The average length of life of the 15 adult males was 14.1 days; of the 21 females, 14.9 days.

Copulation takes place soon after the adult emergence, and several days elapse before egg laying commences. In one instance this period lasted for 17 days, but this is apparently abnormal. Table 2 summarizes the preoviposition period of a few females in 1918 and 1919.

TABLE 2.—*Preoviposition period of the eye-spotted bud moth, 1918 and 1919*

| Year | Number of females | Date adults emerged | Date first eggs were laid | Length of period (days) |
|---|---|---|---|---|
| 1918 | 1 | June 3 | June 10 | 7 |
| 1918 | 2 | June 5 | June 10 | 5 |
| 1918 | 1 | June 8 | June 25 | *17 |
| 1919 | 1 | June 2 | July 5 | 3 |
| 1919 | 1 | June 3 | July 5 | 2 |
| 1919 | 1 | June 4 | July 6 | 2 |
| 1919 | 1 | June 5 | July 7 | 2 |
| 1919 | 2 | June 6 | July 7 | 1 |
| 1919 | 1 | June 6 | July 8 | 2 |
| 1919 | 2 | June 7 | July 9 | 2 |
| 1919 | 1 | June 7 | July 10 | 3 |
| 1919 | 1 | June 8 | July 11 | 3 |
| 1919 | 1 | June 8 | July 12 | 4 |
| 1919 | 2 | June 9 | July 10 | 1 |
| 1919 | 2 | June 9 | July 11 | 2 |
| 1919 | 1 | June 11 | July 19 | 8 |
| 1919 | 2 | June 12 | July 16 | 4 |

* Abnormal.

NOTE.—The average length of the preoviposition period for the 23 females studied was 3.6 days.

## OVIPOSITION

The egg-laying activities of the moths were observed many times. As the time for oviposition approaches, there are indications of excitement on the part of the female followed by periods of quietness, during which the tip of her abdomen touches the surface upon which she rests. When ready to oviposit the female lifts the tip of her abdomen and spreads her genitalia so as to expose the opening of the oviduct. Almost immediately a glistening white egg appears at the opening of the ovipositor. The tip of the abdomen is then quickly lowered to the surface of the leaf, and an egg is deposited. The abdomen is raised again, and, after half a minute, or possibly longer, a second egg appears. Several eggs are thus laid in succession. In the case of one female observed, 11 eggs were laid in about 30 minutes. In all cases oviposition occurred at night.

The eggs are laid singly or in overlapping patches of from 2 to 20. They are fastened to the leaf with a viscous material which soon hardens on exposure to the air. This cementlike substance does not dissolve when the eggs are preserved in alcohol. The margin of the egg is firmly attached to the leaf while the center bulges slightly to accommodate the yolk and protoplasm.

The number of eggs laid by a single female varies considerably. The smallest number of eggs laid by a single moth was 28 and the greatest number 450. A record of the number of eggs laid by a group of moths in 1920 and 1921 is summarized in Tables 3 and 4.

TABLE 3.—*Oviposition and number of eggs laid by the eye-spotted bud moth, 1920*[a]

| June | | | | | | | | | | | | | | | | | | | July | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 1 | 2 | 3 | 4 | 5 | Number of eggs laid |
| ♀ | 0 | 0 | 78 | 10 | 46 | 0 | 0 | 0 | (?) | (?) | 4 | ♀ | 0 | 0 | 0 | 0 | ♂ | --- | --- | --- | --- | --- | --- | --- | 138 |
| ♀ | 0 | 0 | 18 | ♂45 | 0 | 0 | (?) | 31 | (?) | (?) | 0 | ♀ | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 94 |
| ♀ | 0 | 0 | 28 | 42 | 0 | 25 | (?) | 53 | (?) | (?) | 8 | 10 | 0 | 0 | 0 | 0 | ♂2 | 0 | 0 | ♀ | --- | --- | --- | --- | 168 |
| ♀ | 0 | 0 | 85 | 27 | 0 | 6 | (?) | 26 | (?) | (?) | 1 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 13 | 167 |
| ♀ | 0 | 35 | 49 | 14 | 0 | 50 | (?) | 27 | (?) | (?) | 0 | ♀ | 0 | 0 | 0 | 0 | ♂ | --- | --- | --- | --- | --- | --- | --- | 175 |
| ♀ | 0 | 19 | 53 | 31 | 0 | 10 | (?) | 18 | (?) | (?) | ♂ | ♀ | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 131 |
| --- | --- | ♀ | 0 | 8 | 0 | 63 | (?) | 50 | (?) | (?) | 11 | 0 | 0 | 0 | 0 | 0 | ♀ | --- | --- | --- | --- | --- | --- | --- | 132 |
| --- | --- | ♀ | 0 | 0 | 0 | 62 | (?) | 32 | (?) | (?) | 17 | ♀ | ♂ | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 111 |
| --- | --- | ♀ | 0 | 0 | 0 | 85 | (?) | 34 | (?) | (?) | 30 | 0 | 0 | 0 | 0 | 3 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 152 |
| --- | --- | ♀ | 0 | 0 | 0 | 64 | (?) | 51 | (?) | (?) | 24 | 14 | (?) | (?) | (?) | 26 | ♂ | 0 | 0 | ♀ | --- | --- | --- | --- | 179 |
| --- | --- | ♀ | 0 | 0 | 0 | 63 | (?) | 37 | (?) | (?) | 8 | 10 | 0 | 0 | 0 | 7 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 125 |
| --- | --- | ♀ | 0 | 0 | (?) | 92 | (?) | 46 | (?) | (?) | 19 | 4 | 0 | 0 | 0 | 0 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 161 |
| --- | --- | ♀ | 0 | 0 | 0 | 6 | (?) | 72 | (?) | (?) | 34 | 5 | 0 | 0 | 0 | 13 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 130 |
| --- | --- | ♀ | 0 | 87 | 0 | 31 | (?) | 50 | (?) | (?) | 15 | 5 | ♀ | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 188 |
| --- | --- | ♀ | 0 | 0 | 0 | 50 | (?) | 31 | (?) | (?) | 11 | 8 | 0 | 0 | (?) | 16 | ♂ | 0 | 0 | ♀ | --- | --- | --- | --- | 116 |
| --- | --- | ♀ | 0 | 0 | 0 | 0 | (?) | 106 | (?) | (?) | 10 | 0 | 0 | 0 | 0 | 8 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 124 |
| --- | --- | ♀ | 0 | 0 | 0 | 6 | (?) | 70 | (?) | (?) | 38 | 17 | 0 | (?) | (?) | 29 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 160 |
| --- | --- | ♀ | 0 | 51 | 0 | 11 | (?) | 80 | (?) | (?) | 13 | 2 | 0 | 0 | 0 | 8 | ♂ | 0 | ♀ | --- | --- | --- | --- | --- | 165 |
| --- | --- | --- | --- | ♀ | 0 | 0 | (?) | 67 | (?) | (?) | 11 | 16 | 0 | 0 | (?) | 24 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 118 |
| --- | --- | --- | --- | ♀ | 0 | 15 | (?) | 86 | (?) | (?) | 0 | 17 | 0 | 0 | (?) | 23 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 141 |
| --- | --- | --- | --- | --- | ♀ | 0 | (?) | 25 | (?) | (?) | 13 | 32 | 0 | 0 | 0 | 8 | ♂ | ♀ | --- | --- | --- | --- | --- | --- | 78 |

[a] ♂ and ♀ at left of chart indicate dates when male and female moths emerged and those at right of chart indicate dates when the males and females died. No observation was made on days marked (?). The eggs were counted the following day.

NOTE.—There were 21 pairs of adults under observation, and the total number of eggs laid was 2,953.

TABLE 4.—*Oviposition and number of eggs laid by the eye-spotted bud moth, 1921* •

| June | | | | | | | | | | | | | | | | | | | | | | | | | | | | | July | | | Number of eggs laid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 1 | 2 | 3 | |
| ♂ | 0 | 0 | 110 | 49 | 35 | 31 | 15 | 20 | 21 | 19 | 8 | 13 | (?) | 27 | 8 | 8 | 0 | 11 | 0 | 0 | 11 | ♀ | | | | | | | | | | 386 |
| | ♂ | 0 | 128 | 44 | 39 | 30 | 22 | 13 | 31 | 19 | 23 | 19 | (?) | 26 | 15 | 10 | 8 | 3 | 0 | 0 | 20 | ♀ | | | | | | | | | | 450 |
| | | ♂ | | 0 | 95 | 43 | 26 | 29 | 14 | 26 | ♀ | | | | | | | | | | | | | | | | | | | | | 233 |
| | | ♂ | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ♀ | 0 | 0 | 29 | 0 | 0 | 0 | ♂ | 0 | | | | | | | | | | | | | 29 |
| | | | ♂ | 0 | 0 | 0 | 0 | 0 | 0 | ♀ | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | (?) | 18 | 0 | 0 | ♀ | | | | | | | | 28 |
| | | | ♂ | 0 | 68 | 23 | 19 | 0 | 7 | 20 | 6 | 13 | (?) | 14 | 5 | 9 | 4 | 0 | 0 | 0 | ♂ | | | | | | | | | | | 188 |
| | | | ♂ | 0 | 54 | 13 | 22 | 34 | 6 | 0 | 9 | 3 | (?) | 15 | ♂ | | | | | | | | | | | | | | | | | 156 |
| | | | | ♂ | 2 | 11 | 9 | 41 | 20 | 8 | 3 | 0 | (?) | 26 | 11 | 18 | 14 | 0 | 0 | 0 | 15 | 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 8 | ♀ | 192 |
| | | | | ♂ | 0 | 8 | 47 | 29 | 33 | 32 | 0 | 23 | 9 | 0 | 0 | 0 | ♀ | | | | | | | | | | | | | | | 181 |
| | | | | ♂ | 0 | 0 | 0 | 67 | 29 | 42 | 0 | 21 | (?) | 0 | ♀ | | | | | | | | | | | | | | | | | 159 |
| | | | | | ♂ | 0 | 79 | 6 | 62 | 13 | 0 | 3 | 0 | ♂ | | | | | | | | | | | | | | | | | | | 163 |
| | | | | | ♂ | 0 | 56 | 0 | 45 | 18 | 0 | {♂ 11 / ♀} | (?) | 45 | 6 | 9 | 6 | 0 | 0 | 0 | 0 | ♀ | | | | | | | | | | | 196 |
| | | | | | ♂ | 0 | 0 | 0 | 4 | 82 | 2 | 0 | (?) | 26 | 0 | 4 | ♂ | 0 | 0 | 0 | 0 | ♀ | | | | | | | | | | | 118 |
| | | | | | ♂ | 0 | 0 | 0 | 0 | 76 | 2 | 0 | (?) | 113 | 0 | 32 | ♂ | 0 | 0 | (?) | 31 | 5 | 0 | 0 | 0 | ♀ | | | | | | | 259 |
| | | | | | | ♂ | 0 | 0 | 53 | 30 | 0 | 30 | 32 | 10 | 0 | 17 | 0 | 0 | 0 | (?) | 15 | 0 | 0 | ♀ | | | | | | | | | 187 |
| | | | | | | ♂ | 0 | 0 | 37 | 88 | 0 | 47 | 33 | 19 | 16 | 15 | 8 | 0 | 0 | 0 | ♀5 | | | | | | | | | | | 268 |
| | | | | | | | ♂ | 0 | 0 | 77 | 13 | 0 | (?) | 82 | 0 | 22 | ♂ | 0 | 0 | 0 | ♀ | | | | | | | | | | | 194 |
| | | | | | | | ♂ | 35 | 46 | 46 | 12 | 0 | (?) | 71 | 0 | {♂ 11 / ♀} | 0 | 8 | ♀ | | | | | | | | | | | | | 229 |
| | | | | | | | | ♂ | 0 | 0 | 57 | 0 | (?) | 95 | 0 | 29 | 0 | 0 | 0 | 0 | 3 | ♂3 | | | | | | | | | | 187 |
| | | | | | | | | | | ♂ | 0 | 0 | (?) | 129 | 0 | 51 | 12 | 0 | 0 | 0 | ♂ | 29 | ♀ | | | | | | | | | 221 |
| | | | | | | | | | | ♂ | 0 | 0 | 0 | 72 | 0 | 28 | ♂ | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 1 | ♀ | | | | | 109 |

• ♂ and ♀ at left of chart indicate dates when male and female moths emerged; at the right of chart they indicate dates when male and females died. (?) indicates that no records were made.

NOTE.—The total number of eggs laid by females of 21 pairs of moths was 4,133.

## THE EGG

The egg measures 0.8 mm. in length by 0.6 mm in width (pl. 2, A). It is much flattened or scalelike, oval in shape, and bluntly pointed at one end. This pointedness is due to the pressure of the ovipositor at the time of deposition. Sometimes the egg is drawn out into a fine hairlike point at one end, but this is not characteristic. It is transparent, milky, and granular, and is more granular at the rounded end. Its contents are surrounded by a thin, almost invisible, vitelline membrane. Outside of this is a rather tough, transparent chorion faintly sculptured with polygonal markings.

The incubation period of the eggs varies from 7 to 12 days. In 1918 it lasted from 7 to 9 days, the average being 8.1 days. A record kept in 1919 on a total of 986 eggs showed that the egg stage lasted from 8 to 12 days, the average being 9.1 days. The data covering these observations are shown in Tables 5 and 6.

TABLE 5.—*Incubation period of eggs in 1918*

| Date eggs laid | Date eggs hatched | Incubation period (days) |
|---|---|---|
| June 10 | June 17 to 18 | 7 to 8. |
| June 9 to 13 | June 17 to 18 | 8 to 9. |
| June 10 | June 17 | 7. |
| June 9 | June 17 to 18 | 8 to 9. |

TABLE 6.—*Incubation period of eggs in 1919*

| Number of eggs | Date eggs laid | Date eggs hatched | Incubation period (days) |
|---|---|---|---|
| 95 | June 5 | June 14 | 9 |
| 200 | June 7 | June 15 to 17 | 8-10 |
| 88 | June 8 | June 16 to 17 | 8- 9 |
| 174 | June 9 | June 17 | 8 |
| 47 | June 8 to 9 | June 16 to 17 | 8 |
| 130 | June 9 to 10 | June 17 to 18 | 8 |
| 122 | June 11 | June 19 to 21 | 8-10 |
| 62 | June 12 | June 20 to 21 | 8- 9 |
| 34 | June 13 | June 21 | 8 |
| 6 | June 18 | June 28 | 10 |
| 2 | June 19 | June 27 | 8 |
| 20 | June 20 | June 30 to July 1 | 10-11 |
| 5 | June 22 | July 3 | 11 |
| 1 | June 24 | July 4 | 10 |
| Total... 986 | Average length of incubation period.. | | 9.1 |

In the course of a few hours after the egg is laid the yolk becomes pale yellow or straw color and many interesting changes take place which can be followed by means of a hand lens or microscope and illumination from transmitted light. The flatness and thinness of the eggs made it possible to observe the grosser embryonic changes. The eggs were obtained by inducing the females to oviposit on microscope slides. These were examined under the low power of a compound microscope, and numerous observations were made at short intervals of all movements and changes within the eggs. Photomicrographs and diagrams were made illustrating the important changes.

Almost immediately after oviposition, distinct pulsating movements of the yolk are noticeable within the egg. These movements are caused by the migration of the yolk cells toward the opposite ends of the egg (pl. 2, B). During the first few hours, before the yolk gathers at the opposite ends, the blastoderm forms at the larger end of the egg and pushes into the center. This can not be observed in the living egg because the yolk obscures the germ band. Furthermore, the comparative thickness of the egg prevents a view of a single layer of cells. If the egg is placed in weak Flemming's fluid, however, the young embryo can be seen before the yolk moves to the ends of the egg. The first changes are very rapid, and it would be necessary to resort to sectioning to reveal the formation of the blastoderm.

In the course of 10 or 12 hours the yolk gathers in two semicircular-shaped bodies at the opposite ends of the egg, and in the living egg the embryo becomes visible for the first time (pl. 2, C). The embryo at this time is rounded or slightly kidney-shaped. It consists of two portions, an inner densely granular portion and a less granular outer portion.

The yolk soon commences to flow, by irregular pulsations, about the embryo, and at the end of 24 to 48 hours completely surrounds the embryo (pl. 2, D). Division membranes appear in the yolk, giving it a segmented appearance. The outline of the embryo at this time is not as regular as before, due to the segmented condition of the yolk. A slight shrinking of the contents of the egg and the

pulling of the vitelline membrane away from the edge of the chorion likewise becomes visible.

Approximately 54 hours after oviposition the legs appear as three invaginations in the yolk mass (pl. 2, E). The shape of the embryo becomes more pronounced and the contents of the egg shrink distinctly from the edge of the chorion. The segmentation of the embryo is not visible at this time.

About four days after oviposition the embryo becomes segmented, and the legs are well formed and tightly pressed against the under side of the thorax, which at this stage of development is turned toward the outside of the egg (pl. 2, F). The mouth parts commence to form, and the first indication of eyes is represented by two small reddish spots on opposite sides of the head, one lying directly below the other.

Five days after oviposition the embryo makes a revolution in a horizontal plane (pl. 2, G). This is brought about by the elongation of the embryo and the turning of the tip of the abdomen backward. The legs are thus thrown between the thorax and the abdomen. At this time the mouth parts are very distinct, and a dark line appears close to the exterior wall of the embryo, running from the posterior edge of the thorax to about the middle of the abdomen. This line is probably the mid-intestine. The eye spots and segmentation of the embryo are likewise very prominent. The abdomen, due to its new position, lies straight and is not curled at the tip as before.

In a short time, usually less than 24 hours, a second revolution takes place in a vertical direction which brings the legs under the body. The head and thorax are then turned so that one sees them from the dorsal aspect, both eye spots thereby becoming visible (pl. 2, H). The abdomen elongates and becomes curved again at the tip (pl. 2, I). At the same time the yolk supply is rapidly consumed and the contents of the egg shrink noticeably from the edge of the chorion.

On the tenth or eleventh day the embryo becomes mature and the egg is ready to hatch. The head and anterior margin of the prothoracic shield become chitinized. The supply of yolk is entirely consumed and the embryo occupies all the space within the vitelline membrane (pl. 2, J). The embryo finally emerges by cutting a slit through the chorion with its mandibles (pl. 2, K).

## THE LARVA

The newly hatched larvae are pale yellow in color, but as soon as the first molt occurs they turn deep yellow, and later pinkish brown. It is not until after the second molt that they assume the deep-brown color characteristic of the mature larva (fig. 1, A). The head and prothorax in all instars are shiny black except immediately after a molt, when they are white or pale gray. Setal maps are shown in Figure 1, E, F, G, H.

The bud-moth larvae resemble somewhat the larvae of *Mineola indigenella* and *Sparganothis idaeusalis* which also attack the apple. The former is similar in color but when full grown is larger and has a very much roughened head. The latter is slightly paler, has a light-brown head and prothoracic shield, is much more active than the eye-spotted bud moth, and does not inclose itself in a feeding tube.

In all cases observed the eggs were laid on the upper side of the leaves, and the larvae on hatching were found on this side. This surface is smooth and does not afford the protection of the under, hairy side. Consequently it was not surprising to find that newly hatched larvae in the laboratory, as well as those placed on the leaves in the orchard, soon migrated to the under side of the leaves. Observations made on 793 larvae, with respect to the side of the leaf preferred by them, are represented in Figure 2.

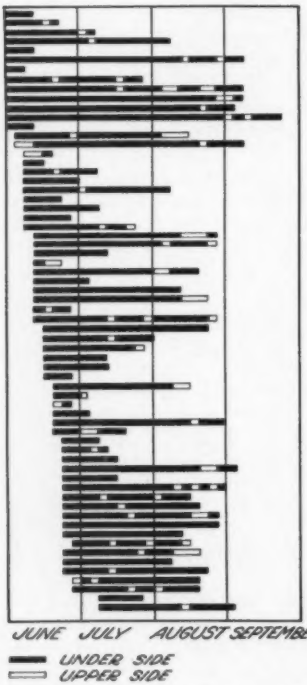



FIG. 2.—Diagram illustrating the position of bud-moth larvae on the under and upper sides of the leaf

## LARVAL CASES

The newly hatched larvae begin at once to spin silk. At first only a few threads are spun to enable them to maintain their position on the foliage against wind and rain. Later leaves are tied together to form protective shelters inside of which the larvae construct their feeding tubes. These feeding tubes are begun about 24 hours after hatching and are quite compact. At first they are formed on the under side of the leaves usually along the midribs or larger veins. They are white in color and made of silk in which are incorporated plant hairs from the under side of the leaf. They are quite inconspicuous and are readily overlooked. At the end of the first day such cases are about one-sixteenth of an inch long. Two days later they measure about three-sixteenths of an inch (pl. 1, B).

Larvae begin to feed two days after hatching and, as they continue to take food, the frass is ejected from one end of the case and added to the exterior. The outside of the feeding tube soon becomes gray or black as the result of the accumulation of fecula (pl. 1, D). The inner silken layer of the tube, however, remains white or of a slightly creamy color.

The shape of the feeding tubes varies considerably. Some are small tubular shelters almost closed at one end and enlarged at the opposite end. Others are straight tubes open at both ends. Still others are tubular but are covered with a tentlike sheet of silk. The larvae seldom wander from these but sometimes abandon them and construct new ones. The cases are lengthened as the larvae increase their feeding grounds.

## FEEDING HABITS

The larvae are shallow feeders on both foliage and fruit. When feeding on the under surface of a leaf they make holes through the lower epidermis and parenchyma as far as the upper epidermis. When feeding from the upper side they rarely eat through the lower epidermis. Nevertheless they consume a considerable quantity of plant tissue, and as they do not eat single large areas but nibble here and there, it is easy to understand how they become so injurious. A record of the measurements of leaf area eaten by the larvae is given in Table 7.

TABLE 7.—*Amount of leaf tissue eaten by eye-spotted bud moth larvae*

| Number of larvae | Date feeding began | Date feeding ceased | Feeding period (days) [a] | Leaf surface removed (square inches) |
|---|---|---|---|---|
| 1 | July 3 | Aug. 7 | 35 | 1 |
| 3 | July 5 | July 13 to Nov. 9 | 232 | 11 1/6 |
| 7 | July 8 | July 21 to Oct. 28 | 373 | 18 1/2 |
| 2 | July 10 | July 26 to Aug. 3 | 39 | 3 1/6 |
| 2 | July 15 | Sept. 28 to Oct. 10 | 235 | 11 7/8 |
| 4 | July 17 | Aug. 3 to Nov. 20 | 280 | 10 5/6 |
| 1 | July 11 | July 26 | 15 | 1 3/4 |
| 1 | July 20 | Oct. 10 | 82 | 3 1/6 |
| 1 | July 26 | Oct. 22 | 88 | 4 1/4 |
| 1 | Aug. 3 | Oct. 21 | 79 | 3 1/4 |
| 1 | Aug. 8 | Oct. 10 | 63 | 1 1/2 |
| 1 | Aug. 28 | Oct. 10 | 43 | 1 5/8 |

[a] Figured for the number of larvae feeding on these dates.

The feeding habits of the larvae from midsummer till late fall are very irregular. In most cases they cease feeding entirely and go into a quiescent state. Occasionally quiescent larvae become active and start feeding again. A summary of late summer and fall activities of a number of larvae is given in Table 8.

TABLE 8.—*Late summer and fall activities of eye-spotted bud moth larvae*

| Number of larvae | Date feeding ceased | Date larva entered resting period | Date feeding resumed | Date larva entered resting period |
|---|---|---|---|---|
| 1 | July 17 | July 17 | | |
| 1 | July 18 | July 21 | | |
| 1 | July 20 | July 20 | | |
| 1 | July 20 | July 23 | July 27 | Aug. 8. |
| 1 | July 21 | July 21 | | |
| 1 | Aug. 3 | Aug. 8 | | |
| 1 | Aug. 15 | Aug. 26 | | |
| 1 | Aug. 28 | Aug. 28 | | |
| 1 | Sept. 6 | Sept. 6 | | |
| 1 | Sept. 26 | Sept. 26 | | |
| 1 | Oct. 3 | Oct. 3 | | |
| 1 | Oct. 9 | Nov. 21 | | |
| 1 | Oct. 10 | Nov. 25 | | |
| 1 | Oct. 22 | Oct. 22 | | |
| 1 | Oct. 28 | Nov. 22 | | |
| 2 | Nov. 9 | Nov. 9 to Nov. 21 | | |
| 1 | Nov. 25 | Nov. 26 | | |

Larvae of the eighth, ninth, or tenth instars hibernate in little silken cases on the twigs near the buds. In confinement, many of them burrowed into the cork stoppers of the rearing bottles or into the petioles of leaves, but some formed no hibernacula at all.

## NUMBER OF MOLTS

The number of instars and the molting activities of the larvae were discussed by the writer [3] in 1922. It is very evident that the number varies with the latitude. Sanders (1919) [4] found that there are seven molts in Canada. Porter (1924) [5] records only six for Connecticut. In Pennsylvania there are usually 11 molts, but in some cases 12 molts occur. Overwintering larvae may molt two or three times in the spring after resuming activities. A summary of the number of molts and the average length of the different instars is given in Table 9. It will be noticed that the length of the instar increases with each successive molt.

TABLE 9.—*Length of the larval instars of the eye-spotted bud moth, summer, 1918, and spring, 1919*

| Length of instars (days) | Number of larvae | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | First instar | Second instar | Third instar | Fourth instar | Fifth instar | Sixth instar | Seventh instar | Eighth instar | Ninth instar | Tenth instar | Eleventh instar | Twelfth instar |
| 2 | 1 | | | | | | | 5 | | | | |
| 3 | | | | | 1 | | | | | | | |
| 4 | 2 | 1 | 6 | | 2 | | | | | | | |
| 5 | 20 | 2 | 4 | 1 | 1 | | 1 | | | | | |
| 6 | 29 | 14 | 2 | 1 | | 2 | | | | | | |
| 7 | 2 | 12 | 6 | 3 | 1 | 2 | | | | | | |
| 8 | 3 | 13 | 12 | 5 | | | 2 | | | 1 | | |
| 9 | 4 | | 2 | 4 | 2 | | 1 | | | | | |
| 10 | | 2 | 4 | 4 | 2 | | 6 | | 1 | | | |
| 11 | | 1 | | 2 | 4 | 1 | 2 | 2 | 2 | | | |
| 12 | 2 | | 6 | 3 | 2 | 2 | 2 | 1 | | | | |
| 13 | | | | 5 | 4 | 2 | | 1 | | | | |
| 14 | | | | 5 | 2 | 2 | 2 | 1 | | | | 1 |
| 15 | | | | 1 | 1 | 2 | 3 | 4 | 2 | 1 | | 1 |
| 16 | | | | 2 | 4 | 1 | | 2 | 1 | 1 | 4 | |
| 17 | | | | 1 | 2 | 1 | 1 | 1 | | | | |
| 18 | | | | 1 | 1 | | | 1 | 3 | 2 | | |
| 19 | | | 1 | | 1 | 2 | | 1 | | | | |
| 20 | | | | | 1 | 3 | 1 | | 1 | | | |
| 21 | | | | 1 | 1 | 1 | | 1 | | | | |
| 22 | | | | | | | 1 | | | | | 1 |
| 23 | | | | | | 1 | | | | | 2 | 3 |
| 24 | | | | | | | | 2 | | | | |
| 25 | | | | | | | 1 | | 1 | | | |
| 26 | | | | | | 1 | | | | | | 1 |
| 27 | | | | | | 1 | | | | 1 | | |
| 28 | | | | | | | 1 | 1 | | 1 | | |
| 29 | | | | | | | | | | | 1 | |
| 30 | | | | | 1 | | | | | 1 | | |
| 32 | | | | | | | | | | | 1 | |
| 33 | | | | | | 1 | | | 1 | 1 | | 1 |
| 38 | | | | | | | | | | | 1 | |
| 39 | | | | | | | | | | 1 | | |
| 42 | | | | | | | | 1 | 1 | | | |
| 49 | | | | | | | | | 1 | | | |
| Average length of instars (days) | 6.1 | 7.0 | 7.9 | 11.3 | 13.0 | 16.2 | 13.3 | 14.8 | 21.5 | 23.2 | 26.8 | 22.3 |
| Total number of larvae | 63 | 45 | 43 | 30 | 33 | 25 | 24 | 24 | 14 | 10 | 9 | 8 |

## LARVAL MORTALITY

The larvae are very susceptible to injury in confinement and the mortality was unusually high. Possibly similar conditions would be found with larvae under natural conditions. In breeding cages the mortality was higher during the earlier instars. This is well illustrated in Table 10.

[3] FROST, S. W. ECDYSIS IN TMETOCERA OCELLANA SCHIFF. Ann. Ent. Soc. Amer. 15: 164-168, illus. 1922.
[4] SANDERS, G. E., and DUSTAN, A. G. Op. cit.
[5] PORTER, B. A. Op. cit.

TABLE 10.—*Mortality of bud-moth larvae during different instars*

| Instar | Dead or missing | Instar | Dead or missing |
|---|---|---|---|
| First | 26 | Seventh | 5 |
| Second | 49 | Eighth | 2 |
| Third | 17 | Ninth | 2 |
| Fourth | 6 | Tenth | 1 |
| Fifth | 3 | | |
| Sixth | 0 | Total | 111 |

The number of dead and missing larvae in breeding cages in which approximately 1,000 larvae were handled during 1918 and 1919 was as shown in Table 11.

TABLE 11.—*Occurrence of dead and missing larvae in captivity*

| | 1918 | | | | | | | | 1919 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Total | June | July | Aug. | Sept. | Oct. | Total |
| Dead | 8 | 14 | 1 | 2 | 3 | 4 | 0 | 32 | 18 | 21 | 4 | 1 | 1 | 45 |
| Missing | 10 | 14 | 7 | 2 | 0 | 0 | 0 | 33 | 38 | 24 | 1 | 1 | 2 | 66 |
| Injured | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 3 | 0 | 4 | 1 | 1 | 0 | 6 |

## THE PUPA

The pupa varies from 7.1 mm. to 8 mm. in length, and is brownish in color (fig. 1, F). It is quite slender and the abdomen tapers gradually toward the posterior end, terminating in a blunt or rounded segment without a cremaster. The dorsal aspect of the abdominal segments bears two rows of spines; a row of strong spines on the anterior margin, and a row of weaker spines across the middle of each segment. The last segment bears about 10 strong spines at the tip, on the dorsal side. There are in addition eight weak hooks, four extending from the dorsal side and four from the ventral surface. The latter are arranged in pairs on each side of the anal opening. The labrum is set off by distinct sutures. The maxillary palpi are about twice as long as the labial palpi and completely inclose them. The femora of the prothoracic legs extend slightly beyond the tips of the maxillary palpi. The prothoracic legs extend half way to the edge, the mesothoracic legs nearly to the edge, and the metathoracic legs slightly beyond the edge of the wing pads. The antennae are comparatively long, reaching nearly to the edge of the wing pads.

Transformation to the pupal stage occurs three or four days after the coccoons are spun. Usually it occurs in the spring. Occasionally, however, a few individuals may transform in late summer when a partial second generation is produced. Pupae are usually found concealed among dried leaves. Before the adults emerge they work themselves part way out of the cocoons. Tables 12 and 13 give the duration of the pupal period from 1918 and 1919.

TABLE 12.—*Length of pupal period, 1918*

| Number of larvae | Date of pupation | Date of emergence of adults | Length of pupal period (days) |
|---|---|---|---|
| 1 | May 20 | May 30 | 10 |
| 2 | May 20 | June 1 | 12 |
| 2 | May 22 | June 3 | 12 |
| 1 | May 28 | June 4 | 7 |
| 1 | May 28 | June 5 | 8 |
| 2 | May 28 | June 6 | 9 |
| 1 | May 28 | June 7 | 10 |
| 2 | May 28 | June 10 | 13 |

NOTE.—The average length of the pupal period for the 12 larvae studied was 10.5 days.

TABLE 13.—*Length of pupal period, 1919*

| Number of larvae | Date of pupation | Date of emergence of adults | Length of pupal period (days) |
|---|---|---|---|
| 1 | May 29 | June 2 | 4 |
| 1 | May 29 | June 4 | 6 |
| 1 | May 29 | June 5 | 7 |
| 2 | May 29 | June 6 | 8 |
| 4 | May 29 | June 7 | 9 |
| 1 | May 29 | June 9 | 11 |
| 3 | May 30 | June 3 | 4 |
| 2 | May 30 | June 5 | 6 |
| 2 | May 30 | June 6 | 7 |
| 1 | May 30 | June 7 | 8 |
| 1 | May 30 | June 16 | 17 |
| 1 | June 2 | June 12 | 10 |
| 1 | June 2 | June 15 | 13 |
| 1 | June 2 | June 17 | 15 |
| 1 | June 2 | June 28 | 26 |

NOTE.—The average length of the pupal period for the 23 larvae studied was 9 days.

## NUMBER OF GENERATIONS

Normally there is but one complete generation of the eye-spotted bud moth in Pennsylvania. Observations made in 1918 show a partial second generation. Larvae which hatched from eggs laid about the middle of June continued their feeding until fall. The majority entered hibernation in the eighth and ninth instars and completed their development the following spring, but a few pupated in August and adults issued and laid eggs. During the seasons of 1919, 1920, and 1921, only a single brood occurred. It appears from several years' observations that a single brood is normal.

## PARASITES AND PREDATORS

The parasitic and predacious enemies of the bud moth have been very carefully reviewed by Porter (1924).[6] One species, not mentioned, might be added, namely: *Itoplectis obesus* described by Cushman, 1917.[7] This makes a total of 26 known parasites of the eye-spotted bud moth, 15 of which occur in North America.

[6] PORTER, B. A. Op. cit.

[7] CUSHMAN, R. A. EIGHT NEW SPECIES OF REARED ICHNEUMON-FLIES, WITH NOTES ON SOME OTHER SPECIES. U. S. Natl. Mus. Proc. 53: 457–469. 1917.

A certain mite was frequently found associated with the bud-moth larvae in their feeding cases, but it is questionable whether this mite attacks the bud-moth larvae or simply takes advantage of the shelter offered by the larvae and feeds on excrement or small organisms present.

Several predators have been recorded in literature, namely: A Carabid beetle *Triphleps* sp., a mite *Anystis agilis* Banks, and a mud wasp, *Odynerus Catskillensis* Sauss.

## SUMMARY

The eye-spotted bud moth is occasionally responsible for severe injury to the fruit and foliage of the apple. It was first reported in this country in 1841, and since that time has been injurious in southern Canada and in at least 24 States in the northern part of the United States.

Injury is caused by the larvae which attack the opening buds, foliage, and newly set fruit in the spring, and the mature fruit in late summer. The injury to mature fruit is the more serious in southern Pennsylvania.

Larvae of the eighth, ninth, and tenth instars hibernate in small hibernacula on twigs near the buds. These emerge early the following spring, and after molting two or three times become mature toward the end of May and pupate within curled leaves. The pupa stage varies from 9 to 10 days. Adults live for an average of 14 days after emergence. There is a preoviposition period of 3 or 4 days. Eggs are laid singly or in small batches of from 2 to 20. They are glued to the leaf with a cementlike substance. The larvae appear about mid-June and feed on fruit and foliage until fall, when they hibernate. Most of their feeding is done on the under side of the leaves. There is high mortality among larvae in breeding cages, particularly during the earlier instars. Normally there is one generation of moths a year.

# RELATION OF MATURITY TO THE NUTRITIVE VALUE OF FIRST, SECOND, AND THIRD CUTTINGS OF IRRIGATED ALFALFA[1]

By JERRY SOTOLA[2]

*Assistant Professor and Assistant Animal Husbandman, Washington Agricultural Experiment Station*

## INTRODUCTION

Under conditions of irrigation farming in the Yakima River Valley of Washington three cuttings of alfalfa are usually harvested and produce hay of fine quality. Yields in tons and permanency of stand have been the primary considerations in the production of such hay, only slight emphasis being placed upon nutritive value. Some attention has been given to the stage of maturity (*36*)[3] in its relationship to yield of the three cuttings. Feeding experiments with lambs over a period of four years have shown the three cuttings of alfalfa harvested in one-half bloom to possess equal values for fattening.

Subsequent digestion and metabolism studies reported in full in this paper supply further information pertaining to the nutritive value of the three cuttings, when each is harvested in the one-fourth, one-half, and three-fourths stages of bloom.

## REVIEW OF LITERATURE

A brief survey has been made of literature pertaining to the stage of maturity, chemical composition, and feeding value and palatability of different cuttings of alfalfa.

### STAGE OF MATURITY

Cottrell (*9*), Ten Eyck (*39*), Clarke (*7*) and Coburn (*8*) recommend cutting alfalfa in the one-tenth bloom stage for general purpose hay. McCampbell (*28*), of the Kansas station, found hay from alfalfa cut in full bloom to be most suitable for horses at hard work. On the basis of later work at that station (*34*) it is recommended to harvest first cutting alfalfa in the one-tenth bloom stage and to delay successive cuttings in the same season until the crop reaches full bloom or nearly so.

Foster and Merrill at the Utah station (*11*), Harcourt in Canada (*16*), and Snyder and Hummel (*37*) at the Minnesota station favor the one-third stage of bloom. Freeman (*12*) observes that the one-fifth to one-third stage of bloom is best under Arizona conditions, while Voorhees (*40*) considers that the fourth bloom yields the best quality hay.

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[3] Reference is made by number (italic) to "Literature cited," p. 381.

Hughes (*21*) at the Iowa station suggests cutting alfalfa when one-tenth to one-fourth in bloom, while Hunt (*22*) states that in irrigated regions the practice is to cut when one-tenth to one-third of the total flowers are formed. He adds that the crop should be cut as soon as the lower leaves begin to turn yellow, regardless of the stage of bloom.

Kiesselbach (*23*) at the Nebraska station notes that too frequent cutting results in weakening of alfalfa plants, and recommends the first-bloom stage for best hay. Bennett (*5*) also advises cutting when a few blooms appear over the field. At the Ohio station Williams and Kyle (*43*) observed injury to alfalfa from cutting too early before first bloom or from cutting too late after the crop was too mature. A similar observation is made by Porter and Dynes (*32*) in North Dakota, and Stewart (*38*) also points out that too early cutting is accompanied by too much danger to the stand.

Singleton (*36*) at the Washington station reported for a total of three cuttings average yields of 6.86, 6.71, and 5.83 tons of the three-fourths, one-half, and early-blooming stages, respectively. Cutting off new shoots did not affect the yield or hurt the stand.

Russell and Morrison in Wisconsin (*33*) recommended the near-full-bloom stage for cutting alfalfa without getting the hay too coarse. Early cutting thinned the stand, weakened the plants, and permitted the encroachment of weeds. Cutting off crown shoots, contrary to common opinion, did not injure the stand. Headden (*19*) at the Colorado station also favors the full-bloom stage.

According to Piper (*31*) the general practice in America is to cut alfalfa for hay shortly after the first blossoms appear, except for horses, in which case the full-bloom stage is considered best, as earlier cuttings generally prove too laxative.

At the Michigan station (*35*) Shoesmith states that alfalfa should be cut when it begins to bloom and new shoots appear at the crown, while J. E. Wing (*44*) writes that usually when alfalfa is ready to be cut, it will be partly in bloom, sometimes being much more advanced than at other times. It is further suggested that alfalfa should be cut for hay whenever it is ready to make new growth.

The preceding recommendations favor the early bloom stages, 7 being in favor of the one-tenth bloom, 4 favoring the early bloom, 2 the one-fourth bloom, and 4 the one-third bloom stage. The work of the Wisconsin and Colorado stations appears to be at variance in that the full-bloom stage is favored.

### CHEMICAL ANALYSIS AND DIGESTIBILITY

A survey of the chemical composition of hays of the first, second, and third cuttings of alfalfa was made. Only the averages for 16 determinations chosen from sources reporting three cuttings per year were used. The stage of maturity was not considered in averaging these data, since in many of the investigations just the cutting irrespective of maturity is considered. For comparative purposes all determinations were recalculated to a 93 per cent dry-matter basis, this figure being used because it approximates the moisture content of well-cured hays produced in the Yakima section of the State of Washington. The results of the survey are shown in Table 1.

TABLE 1.—*Average percentage composition of first, second, and third cuttings of alfalfa hay* [a]

[93 per cent dry-matter basis]

| Cutting | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Crude ash |
|---|---|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| First | 14.37 | 31.96 | 35.55 | 1.94 | 9.18 |
| Second | 14.24 | 33.49 | 34.64 | 1.84 | 8.79 |
| Third | 14.21 | 31.15 | 36.64 | 1.93 | 9.07 |

[a] This table was compiled from analyses obtained from the following references: *1, p. 171; 11, p. 168, 171, 174; 13, p. 8; 17, p. 31–32; 18, p. 6, 9; 20, app. Table II; 24, p. 31–37; 25, p. 7; 26, p. 108; 30, p. 16, 31–32; 41, p. 74–75; 42, p. 58.*

Since the average figures do not indicate the variations in composition, the extremes are reported separately. For the first cutting the protein varied from 11.62 to 18.69 per cent; fiber from 26.32 to 42.46 per cent; and the nitrogen-free extract from 27.09 to 39.99 per cent.

The crude protein varied for the second cutting from 11.43 to 17.17 per cent; crude fiber from 26.42 to 41.77 per cent, and nitrogen-free extract from 22.61 to 39.53 per cent. For the third cutting the variation in protein content was from 12.01 to 14.92 per cent; in fiber from 27.29 to 37.47 per cent; and in nitrogen-free extract from 22.61 to 39.53 per cent.

The average figures in Table 1 show very little difference in composition of hays from the three cuttings of alfalfa. A much greater variation is secured in the separate determinations of each cutting.

The average results of a survey of the digestion experiments with hays produced from the three cuttings of alfalfa when these were fed to ruminants are summarized in Table 2.

TABLE 2.—*Average coefficients of digestibility of hay from three cuttings of alfalfa* [a]

| Cutting | Dry matter | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Crude ash |
|---|---|---|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| First | 60.45 | 71.70 | 43.23 | 72.46 | 37.63 | 49.24 |
| Second | 61.79 | 75.90 | 44.42 | 73.86 | 44.32 | 51.91 |
| Third | 55.82 | 68.68 | 38.35 | 68.25 | 39.40 | 44.13 |

[a] The coefficients are averages of those reported in the following publications: *15, p. 26; 20, app. Table II; 24, p. 31–37; 25, p. 28–29; 27, p. 46.*

The variations in coefficients from the different sources are not great. The averages show second-cutting hay to be quite well digested. Markedly lower digestibility for the third-cutting hay can be noticed. From Tables 1 and 2 the content of digestible nutrients in the hays from three cuttings was computed. The results are shown in Table 3.

TABLE 3.—*Average digestible nutrients in first, second, and third cuttings of alfalfa*

| Cutting | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Total digestible nutrients | Nutritive ratio 1 to— |
|---|---|---|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | |
| First | 10.30 | 13.82 | 25.76 | 0.73 | 51.52 | 4.00 |
| Second | 10.81 | 14.88 | 25.59 | .82 | 53.13 | 3.90 |
| Third | 9.76 | 11.95 | 25.01 | .76 | 48.43 | 3.96 |

Table 3 shows that the third-cutting hay contains the least total digestible matter as well as the least digestible crude protein. It forms an excellent basis for later comparisons.

## FEEDING VALUE AND PALATABILITY

### Results with Dairy Cows

At the Utah Station (*6*) the second-crop alfalfa hay proved at least equal to the other crops for milk production. In all cases the cows took more readily to first and third-crop hay than they did to the second-crop hay, and at times were very reluctant about feeding on second-crop hay.

### Results with Sheep

During the years 1922 to 1926 the lamb-feeding experiments at the Washington Irrigation Branch Experiment Station each year included studies with first, second, and third cuttings of northern grown common alfalfa hay, which were made at the one-half bloom stage of maturity. A daily ration of three-fourths of a pound of grain was fed each lamb in addition to all the hay it would consume. During one of these years wheat was substituted for corn. Descriptions of the lambs, the experimental methods, and the three years' results are reported in a previous publication (*14*). The data in Table 4, which are based on four years of field work, fail to show any differences over such a period of time in the feeding results with different cuttings of alfalfa. There was no great difference in condition of the lambs fed on different cuttings when they were ready for market.

TABLE 4.—*Comparative value of first, second, and third cuttings of alfalfa hay in feeding lambs*

[Figures are based upon 60-day experiments for four years]

| Items | Cutting of alfalfa hay | | |
|---|---|---|---|
| | First | Second | Third |
| Number of lambs | 225 | 175 | 175 |
| Initial weight (pounds) | 69.11 | 69.48 | 70.08 |
| Final weight (pounds) | 85.92 | 85.84 | 86.95 |
| Total gain 60 days (pounds) | 16.81 | 16.36 | 16.87 |
| Average daily gain (pound) | .282 | .275 | .283 |
| Average daily ration: | | | |
| Grain fed (pound) | .75 | .75 | .75 |
| Hay fed (pounds) | 2.67 | 2.65 | 2.69 |
| Hay refused (pound) | .49 | .42 | .45 |
| Feed required per cwt. gain: | | | |
| Grain (pounds) | 268 | 275 | 267 |
| Hay fed (pounds) | 953 | 972 | 957 |
| Hay refused (pounds) | 175 | 154 | 160 |
| Hay consumed (pounds) | 778 | 818 | 797 |
| Percentage of hay refused | 18.36 | 15.84 | 16.72 |

The lambs had access to good clean hay. It would naturally be assumed that the most palatable hay would be consumed in greatest quantities. However, Table 4 shows a uniform consumption of all three cuttings, so that the four-year study fails to reveal any great differences in palatability. In fact, had all three lots of lambs been fed the same cutting of hay, they might have been expected to show as great differences as were observed between the groups fed different cuttings.

During the first year's trials two very uniform groups of lambs, each containing 50 head, were fed identical rations, with the result that differences in gains greater than those reported in the experiment covered by Table 4 were observed. The single results of each year show that during two years the feeding gains with second-cutting hay were slightly less than the average for all cuttings. The experiments during one year showed no difference between the feeding gains for second-cutting hay and those for other cuttings, whereas in experiments for another year second-cutting hay showed to best advantage. The results of lamb-fattening experiments with alfalfa fail to support the widespread belief that second-cutting hay is enough lower in nutritive value to be discriminated against in the open market.

### Results with Beef Cattle

Work at the Utah station (*30*) shows that when steers were fed alfalfa with or without grain, the most rapid gains were secured on the early-cut hay and the lowest on the late-cut hay, the rating being as follows: Early cut hay 100, medium-cut hay 77, and late-cut hay 68. A similar rating according to value of cutting for beef production showed first cutting as 100, second cutting 75, and third cutting 110. In respect to palatability as measured by pounds of hay actually eaten, first cutting was rated as 100, second 97, and third, 105. Later work at the same station (*11*) shows that the largest gains by two-year-old steers were made from the early cuttings, and the lowest gains from the late cuttings, the results standing proportionately 100 for early cut hay, 85 for medium-cut hay, and 75 for late-cut hay. The Utah results show the highest feeding value for the third cutting and the lowest for the second, which is in conformity with the earlier work.

Work at Kansas (*34*) shows that the feeding value of alfalfa hay decreases materially as cutting is delayed, the best hay for feeding beef steers being secured at the bud stage and the poorest at the seed stage.

## PURPOSE OF THE INVESTIGATION

This investigation was divided into four phases as follows:

(1) A study of the composition of each of the three cuttings of northern-grown common alfalfa at three stages of maturity was made. Alfalfa of the first, second, and third cuttings was harvested in 1923 and 1924, at the one-fourth, one-half, and three-fourths stages of bloom, these stages being determined as far as possible by bud and blossom counts. In addition to a study of the organic nutrients, studies of calcium and phosphorus were made.

(2) Samples of alfalfa hay of the different cuttings and stages were fed to lambs receiving no other feed. Coefficients of digestibility for each of the nutrients in alfalfa hay were determined, and from these data the digestible nutrient content of the hay of each cutting was computed.

(3) Nitrogen balance studies were undertaken with the view of determining at what stage of maturity of the alfalfa plant the protein is best utilized.

(4) An interpretation of the nutrition studies in terms of acre yield was made.

## METHOD OF EXPERIMENTATION

### GENERAL PROCEDURE

The studies of each stage of maturity of the three cuttings were made in triplicate, two wethers and one ewe being used each time. After a 10-day preliminary period in which the experimental ration was fed, the lambs were put into the metabolism crates for 10 days, quantitative collection of feces and urine was made, and an accurate record of feed, water, and refuse hay kept. At the end of the 10-day experimental period the three lambs on test were given a 10-day rest, the other series of two wethers and one ewe being placed in the crates. In this manner the nine samples of hay collected in 1923 and the nine in 1924 were tested during the spring of 1924. The same lambs used in studying the 1923 samples were fed the hay of 1924 and in the same order. This eliminates the factor of individuality in comparisons of digestibility of similar hays produced in 1923 and 1924.

The age of the lambs within limits is not a great factor, inasmuch as normal lambs between the ages of 6 and 14 months digest feed equally well (*3*). In nutrition experiments it is easier to work with sheep than with cattle, and inasmuch as the results of digestion trials with sheep apply also to cattle in most cases, sheep were used in this test. H. P. Armsby (*3*) states that Wolff, in 1874, compared the results of about 40 German experiments on cattle and sheep, and Jordan and Hall have made similar comparisons of nine American experiments. On the basis of comparisons of this sort it has been generally considered that digestion coefficients obtained with one species of ruminants may be applied to others without material error. The sheep or goat has been the favorite experimental animal. As regards the better grades of roughages, it has been shown that the difference in digestive power between cattle and sheep is not marked

### METHOD OF SAMPLING

All of the hay fed was first chopped into one-half and three-fourths inch lengths and thoroughly mixed and sampled (*10*) for analysis. All the feed for any 10-day experimental period was weighed into paper sacks at one time to equalize moisture changes.

As the trials progressed the refuse hay was carefully collected and sampled. During the study of the 1923 samples some waste hay was collected during each trial, while in subsequent studies with hay of 1924 the lambs ate all the hay without waste.

The metabolism crates were cleaned three times during each 10-day period and the feces of each lamb was collected quantitatively, placed in air-tight receptacles, and the complete sample mixed and sampled for study. The urine was collected daily and kept in stoppered glass flasks over toluol. To prevent loss of ammonia, sulphuric acid instead of toluol was at first added to the samples. However, later study showed this to be unnecessary.

### LAMBS AND THEIR BODY WEIGHTS

Six lambs sired by Hampshire rams and out of Lincoln-Merino ewes were selected from a band of range sheep. The different types were selected with care. Four of the lambs were wethers and two were ewes. The accompanying illustration (fig. 1) gives a fairly accurate idea of type.

These lambs were gradually accustomed to the metabolism crates until they could stand on the half-inch mesh-wire screen with practically no discomfort. The lambs were normal and maintained good appetites throughout the complete series of experiments. During

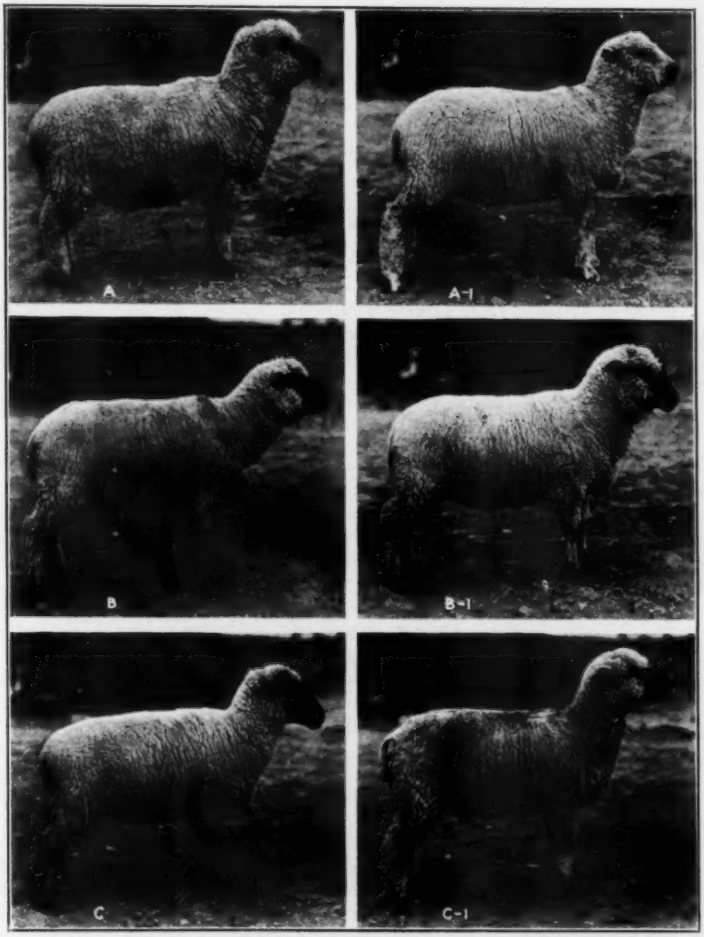


FIG. 1.—The sheep shown in vertical columns are those used in the two series of alfalfa-feeding experiments. The bottom horizontal row shows the ewe lambs, all others being wethers

27 of the 10-day digestion periods the lambs gained weight, whereas they lost weight during 27 other periods. The average loss for the 10-day periods was 2.5 pounds, and the average gain 2.34 pounds per lamb. The average weight of the lambs for the entire period was 82.36 pounds.

### RATIONS FED

The daily ration was fed in three equal portions, the consumption of chopped hay reaching 2.65 pounds (1,200 gm.). This quantity of feed contains 0.20 pounds of digestible crude protein and 1.25 pounds of total digestible nutrients. Such a ration for lambs averageing 82.36 pounds is a medium ration, well above the maintenance requirements. Two grams of salt were added to each feed of hay for which a correction was made. During the preliminary periods free access to salt was given.

The quantity of hay per lamb fed daily does not affect the digestibility (*3*) when alfalfa alone is fed. However, in mixed rations with grains, the heavier rations are generally less digestible.

The lambs drank 2.37 pounds of water for every pound of hay they consumed. This figure includes evaporation. During the warmer months of July and August, the figure was increased to 3.03 pounds of water.

### ANALYTICAL PROCEDURE

Analyses of feed, feces, and urine were made as the experiment progressed, by methods of analysis adopted by the Association of Official Agricultural Chemists (*4*). The phosphorus and calcium content of the hay was also determined.

## CHEMICAL ANALYSIS OF FEED SAMPLES

The chemical composition of hay samples which were fed to the lambs during the digestion trials is reported in a succeeding table based on averages for the two years. Another table stressing the cutting rather than the stage of bloom is added. The calcium and phosphorus content of the samples was determined and is given in Table 7.

The composition of the samples of 1923 indicates that there is no marked difference in the hay at one-fourth and one-half bloom stages. At the more mature stage of three-fourths bloom, the hay contains less protein and is somewhat higher in fiber. The first cutting at the one-fourth bloom stage contained only 29.53 per cent fiber in contrast to 40.23 per cent in the third cutting at the three-fourths bloom stage. The third cutting contained only 8.86 per cent of protein. This particular cutting was harvested late in the season and was produced in cooler weather and seems out of line with the rest of the hays.

The analyses of hay samples of 1924 agree in general with those of 1923. There seems to be no clear-cut relationship between fiber content and maturity in the individual samples. At the one-half and three-fourths bloom stages the second and third cuttings contain more fiber. The third cutting at the three-fourths bloom stage again has a low protein content. Averages of all three cuttings at any particular stage show that in 1924 a progressively lower protein content was found in the hays as maturity progressed. The data for ether extract and ash are quite variable.

The data for the two years have been averaged and are included in Table 5. This table brings out in somewhat sharp relief the fact that the protein content of the hays decreases as the plant matures, while the fiber in turn increases.

TABLE 5.—*Average composition of first, second, and third cuttings of alfalfa hay when the plant is cut at the one-fourth, one-half, and three-fourths stages of bloom* [a]

[Reduced to a 93 per cent dry-matter basis, samples of 1923 and 1924]

| Cutting | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Crude ash |
|---|---|---|---|---|---|
| One-fourth bloom stage: | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| First | 11.78 | 31.96 | 40.16 | 2.20 | 6.90 |
| Second | 13.40 | 35.22 | 34.02 | .97 | 9.40 |
| Third | 13.96 | 36.08 | 33.22 | 1.04 | 8.72 |
| Average | 13.05 | 34.42 | 35.80 | 1.40 | 8.34 |
| One-half bloom stage: | | | | | |
| First | 12.07 | 35.20 | 37.43 | .88 | 7.43 |
| Second | 12.98 | 35.12 | 36.07 | 1.27 | 7.58 |
| Third | 12.37 | 36.21 | 34.98 | 1.22 | 8.23 |
| Average | 12.47 | 35.51 | 36.16 | 1.12 | 7.75 |
| Three-fourths bloom stage: | | | | | |
| First | 11.36 | 34.76 | 38.60 | 1.60 | 6.69 |
| Second | 11.29 | 37.77 | 33.84 | 1.06 | 9.04 |
| Third | 9.06 | 38.59 | 36.42 | .79 | 8.16 |
| Average | 10.57 | 37.04 | 36.29 | 1.15 | 7.96 |

[a] Analysis based on stage of bloom at which hay is cut.

A regrouping of the data contained in Table 5 according to cuttings rather than stages of bloom, is reported in Table 6. The two years' data show that ordinarily the first cutting is somewhat lower in protein than the second and third cuttings at the one-fourth and one-half bloom stages. However, as the alfalfa is permitted to mature the differences become less, and the poorest protein hay is secured with the third cutting made at the three-fourths bloom stage.

TABLE 6.—*Average composition of first, second, and third cuttings of alfalfa hay when the plant is cut at the one-fourth, one-half, and three-fourths stages of bloom* [a]

[Reduced to a 93 per cent dry-matter basis, samples of 1923 and 1924]

| Stage of bloom | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Crude ash |
|---|---|---|---|---|---|
| First cutting: | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| One-fourth | 11.78 | 31.96 | 40.16 | 2.20 | 6.90 |
| One-half | 12.07 | 35.20 | 37.43 | .88 | 7.43 |
| Three-fourths | 11.36 | 34.76 | 38.60 | 1.60 | 6.69 |
| Average | 11.74 | 33.97 | 38.73 | 1.56 | 7.01 |
| Second cutting: | | | | | |
| One-fourth | 13.40 | 35.22 | 34.02 | .97 | 9.40 |
| One-half | 12.98 | 35.12 | 36.07 | 1.27 | 7.58 |
| Three-fourths | 11.29 | 37.77 | 33.84 | 1.06 | 9.04 |
| Average | 12.56 | 36.04 | 34.64 | 1.10 | 8.67 |
| Third cutting: | | | | | |
| One-fourth | 13.96 | 36.08 | 33.22 | 1.04 | 8.72 |
| One-half | 12.37 | 36.21 | 34.98 | 1.22 | 8.23 |
| Three-fourths | 9.06 | 38.59 | 36.42 | .79 | 8.16 |
| Average | 11.80 | 36.96 | 34.87 | 1.02 | 8.37 |

[a] Analysis based on cutting of hay.

The averages for the first, second, and third cuttings in the preceding table when compared with the data of Table 1 show that the hays studied contain somewhat less protein and more fiber than would be expected. Somewhat less ether extract and ash was found in these samples. The calcium and phosphorus content of the same hay samples under discussion was determined. The results of these determinations are contained in Table 7.

TABLE 7.—*Per cent calcium and phosphorus contained in alfalfa hay studied*

[7 per cent moisture basis]

| Cutting | Calcium | | | Phosphorus | | |
|---|---|---|---|---|---|---|
| | 1923 | 1924 | Average | 1923 | 1924 | Average |
| One-fourth bloom: | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| First | 1.371 | 1,234 | 1.302 | 0.164 | 0.145 | 0.154 |
| Second | 1.331 | 1.025 | 1.178 | .203 | .177 | .190 |
| Third | 1.174 | 1.416 | 1.295 | .148 | .218 | .183 |
| Average | 1.292 | 1.225 | 1.258 | .172 | .180 | .176 |
| One-half bloom: | | | | | | |
| First | 1.610 | 1.347 | 1.478 | .162 | .145 | .154 |
| Second | 1.392 | 1.196 | 1.294 | .190 | .169 | .180 |
| Third | 1.354 | 1.546 | 1.450 | .176 | .114 | .145 |
| Average | 1.452 | 1.363 | 1.407 | .176 | .143 | .160 |
| Three-fourth bloom: | | | | | | |
| First | 1.449 | 1.326 | 1.388 | .138 | .165 | .152 |
| Second | 1.352 | 1.189 | 1.270 | .142 | .126 | .134 |
| Third | 1.312 | 1.240 | 1.276 | .093 | .106 | .100 |
| Average | 1.371 | 1.252 | 1.311 | .124 | .132 | .129 |

The calcium content of alfalfa is seven to eleven times greater than its phosphorus content. Plants cut at the one-half bloom stage contain more calcium than similar samples of alfalfa cut at the one-fourth and three-fourths stages of bloom. The data fail to show any significant relationship between the number of the cutting and calcium content. The results with samples of 1923 show that first cutting at any of the stages contains more calcium than the second, and the second more than the third. Results for 1924 show that the first cutting contains more calcium than any of the others at the three-fourths stage of bloom only. At all three stages of bloom that year the second cutting contained less calcium than the third.

The results with phosphorus are quite variable and no conclusions seem warranted in respect to the number of the cutting and phosphorus content. The peculiarly low phosphorus content of the third cutting made at the three-fourths stage of bloom is quite striking in view of the fact that it is also low in protein. Much nutritive value is lost by the hay crop when it is leached by rain or dews. At Ohio (*2*) it was demonstrated that 50 per cent of the nitrogen, 75 per cent of the phosphorus, 90 per cent of the potassium, and 40 per cent of the calcium can be leached out of the hay crop by artificial means.

The calcium and phosphorus content of alfalfa hay is of considerable interest to the feeder. Alfalfa cut at the one-half bloom stage yields most calcium from its three cuttings, while the one-fourth bloom stage is richest in phosphorus. It is recognized that the con-

tent of the mineral elements in any feed is not the only indicator of its value in supplying minerals for body functions. Sunlight and one or more of the vitamins are intimately associated with the utilization of these mineral elements.

## CHEMICAL ANALYSIS OF FECES

The average analysis of the feces from the first 27 digestion trials when hay samples of 1923 were fed are reported separately from the average of the second series of 27 digestion trials, during which period samples of 1924 were fed. For comparative study the figures were computed to the average moisture content of all feces studied. These figures are shown in Table 8.

TABLE 8.—*Average composition of feces when lambs were fed chopped alfalfa hay*

| Series | Water | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Crude ash |
|---|---|---|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Percent* | *Per cent* |
| First | 58.42 | 4.45 | 18.11 | 12.50 | 1.11 | 5.37 |
| Second | 58.42 | 4.25 | 18.72 | 11.45 | 1.86 | 5.30 |

Each lamb excreted on the average 3.05 pounds of feces per day per 100 pounds live weight, when a medium ration of chopped alfalfa hay was fed. For every pound of dry matter actually consumed, 0.47 pound of fecal dry matter was excreted, which is equal to 1.13 pounds of feces, with an average moisture content of 58.42 per cent.

The digestion trials show that an average of 44.79 per cent of the consumed organic matter in the form of alfalfa hay is excreted in the feces. Minimum and maximum figures of 28.91 and 59.88 per cent were noted.

## COEFFICIENTS OF DIGESTIBILITY

When the results of ordinary digestion experiments are corrected for nitrogenous and other excretory products found in the feces, an approximation of true digestibility is obtained. Armsby (*3*) states that the intestinal products found in the feces are, in effect, part of the cost of digesting the feed. He states that they represent the "wear and tear" of the digestive organs. The difference, then, between feed and feces will show the net gain to the animal from the digestion of the feed. From this point of view one may speak of the digestibility as ordinarily determined as the *apparent digestibility*. The subsequent studies are based upon coefficients of apparent digestibility.

The results of a single digestion trial are reported in detail in Table 9. Fifty-four such experiments form the basis of the digestibility studies, but lack of space precludes their tabulation in full. The tables of average coefficients of apparent digestibility bring out in full detail the results of these experiments.

TABLE 9.—*Results of trial 1 secured with wether lamb No. 339. The first cutting of 1923 in the one-fourth bloom stage was fed for 10 days*

| | Dry matter | Crude protein | Crude fiber (N×6.25) | Nitrogen-free extract | Ether extract | Organic matter |
|---|---|---|---|---|---|---|
| Alfalfa hay fed (grams) | 10,646 | 1,427 | 3,380 | 4,675 | 418 | 9,900 |
| Alfalfa hay refused (grams) | 799 | 76 | 341 | 326 | 16 | 749 |
| Total hay consumed (grams) | 9,847 | 1,351 | 3,039 | 4,349 | 402 | 9,151 |
| Feces (grams) | 3,717 | 393 | 1,592 | 1,066 | 74 | 3,125 |
| Total digested (grams) | 6,130 | 958 | 1,447 | 3,283 | 328 | 6,026 |
| Coefficients of digestibility | 62 | 71 | 48 | 76 | 82 | 66 |

Since all the feed for any 10-day digestion trial was weighed into paper sacks at one time, a very accurate determination of the dry-matter intake was possible. The feed rack shown in Figure 2 was so constructed that all feed scattered by the lambs could be recovered. The urine was also collected quantitatively, and its nitrogen content serves as the basis of subsequent nitrogen-metabolism studies.

The coefficients of digestibility represent, by a single number, results of many complex physical and chemical changes. In a subsequent table these coefficients are reported as whole numbers, since even the whole numbers are at best only relative.

The negative coefficients for ether extract signify that the fecal ether extract exceeded that contained in the feed consumed. Only anhydrous ether was used in the determinations. Many of the bile salts are soluble in ether and for that reason even the positive coefficients of digestibility are not reliable. Inasmuch as considerable ash is excreted in the large intestine, fecal ash is made up of indigestible ash as well as excretory ash. For that reason digestion studies with ash are meaningless and are not reported.

The dry matter of first-cutting hay is digested better than the dry matter of either of the other two cuttings, at all three stages of maturity. The protein of the third-cutting hay is not digested as well as that of the first and second cuttings. At the three-fourths bloom stage the digestibility of protein is 30 per cent less than for the other two cuttings, and a considerably lower digestibility of the dry matter and fiber is noted.

The results for 1924 show a lower digestibility for dry matter and crude fiber than do those of 1923. Lambs consumed the 1924 hay completely, while some stems remained in the feed troughs during each trial when the hays of 1923 were fed. The intake of fiber per lamb therefore was greater in the first case and helps explain the somewhat lower digestion coefficients. The coefficients for the third cutting feeding experiments at the three-fourths bloom stage are not as much out of line as those secured for the identical stage produced in 1923.

The results for the two years were averaged and are summarized in Table 10.

The table shows that at the one-fourth and one-half bloom stages there is no marked difference in the digestibility of dry matter. This is true also of the first and second cuttings made at the three-fourths bloom stage. However, the third cutting at the three-fourths bloom stage appears to have a much lower digestibility. A coefficient of 46, which is the average of six determinations, appears to be 9 and 6 points lower than the coefficients for first and second cuttings, respectively.

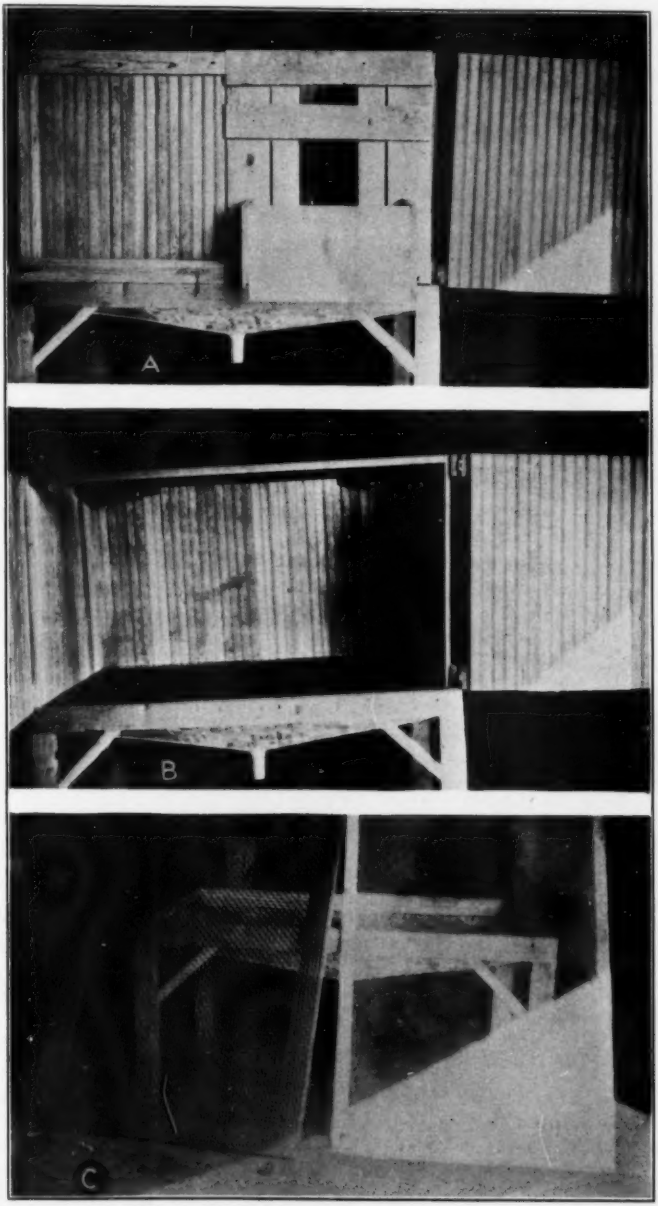


FIG. 2.—A, Metabolism crate with the feed rack in place. B, An open view of the same crate. C, Type of screens used in the construction of these crates

TABLE 10.—*Average coefficients of apparent digestibility secured with irrigated alfalfa hay*

[Produced in 1923 and 1924 a]

| Cutting | Dry matter | Crude protein (N × 6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Organic matter |
|---|---|---|---|---|---|---|
| One-fourth bloom stage: | | | | | | |
| First | 54 | 62 | 40 | 68 | 28 | 57 |
| Second | 55 | 68 | 48 | 63 | −39 | 56 |
| Third | 54 | 67 | 46 | 64 | 4 | 56 |
| Average | 54 | 66 | 45 | 65 | ..... | 56 |
| One-half bloom stage: | | | | | | |
| First | 58 | 66 | 52 | 68 | −38 | 60 |
| Second | 54 | 69 | 44 | 64 | 14 | 56 |
| Third | 53 | 62 | 47 | 67 | ..... | 54 |
| Average | 55 | 66 | 48 | 66 | ..... | 57 |
| Three-quarter bloom stage: | | | | | | |
| First | 55 | 62 | 45 | 67 | 38 | 58 |
| Second | 52 | 60 | 44 | 62 | −6 | 53 |
| Third | 46 | 45 | 38 | 64 | ..... | 46 |
| Average | 51 | 56 | 42 | 64 | ..... | 52 |

a Each stage of maturity was studied in triplicate, so that each coefficient in the table is the average of six determinations.

The digestibility studies with crude protein favor the one-fourth and one-half bloom stages. These appear to produce hay of a protein content that is equally well digested. However, at the three-fourths bloom stage protein of first and second cuttings is similarly digested, the coefficients for these cuttings being 62 and 60, respectively. The third cutting at this stage has a coefficient of only 45. It is true that this third cutting, made so late in the season, is subject to cooler temperature, more humidity during its growth, and a greater leaf loss during its curing period. A tougher fiber seems to develop also, which appears to exert a secondary influence in depressing the digestibility of protein and other nutrients.

The fiber at the one-half bloom stage is better digested than at the one-fourth bloom stage, although the difference is not great. At the three-fourths bloom stage, particularly in the third cutting, the fiber seems to be tougher in nature and lower coefficients are noted. The third cutting which gives a coefficient of 38, ranks last, the first and second cuttings at the three-fourths bloom stage having coefficients of 45 and 44.

The nitrogen-free extract is apparently equally well digested at all three stages of bloom.

## DIGESTIBLE NUTRIENTS OF ALFALFA HAY

The difference in digestible nutrients in the hays of 1923 and 1924 can be explained partly on the basis that some stems were not consumed by the lambs fed the hays produced in 1923, while in subsequent trials the lambs consumed their feed completely. In such cases the waste stems were analyzed and their nutrient content subtracted from the nutrients in the hay fed. The hay actually consumed was more digestible per unit weight than if no stems had

been wasted; however, the waste was small and fairly uniform for each trial, and the relative digestibility of the different samples should not be greatly affected.

A comparison of the two years' results shows a lower digestibility of dry matter in 1924. The third cutting of 1923 made at the three-fourths stage of bloom is shown to possess low nutritive value, being especially low in digestible crude protein and total digestible nutrients. A similar cutting produced in 1924, although rather low in digestible protein, contained as much digestible nutrients as any of the cuttings produced that year.

Averages for all three cuttings produced in 1923 show that hays of almost equal nutritive value are produced at the one-fourth and one-half bloom stages, but that delaying cutting until the three-fourths stage of bloom lowers the nutritive value.

The 1924 average results show that the one-fourth bloom stage of maturity is to be preferred when hays with a high protein content are desired. Progressively less digestible protein is found in the hays produced from plants in the one-half and three-fourths stages of bloom. However, the greatest quantity of total digestible nutrients is secured when the hay is cut at the one-half bloom stage.

The average results (Table 11) based on two years' work show that a high content of digestible crude protein is secured at the one-fourth bloom stage, and a rather lower protein content by permitting the plants to mature. At the one-half bloom stage the plants produce a hay that contains the maximum of digestible nutrients.

TABLE 11.—*Digestible nutrients per 100 pounds when alfalfa is cut at the one-fourth, one-half, and three-fourths bloom stage*

[Average of 1923 and 1924]

| Cutting | Dry matter | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Total digestible nutrients |
|---|---|---|---|---|---|---|
| One-fourth bloom stage: | | | | | | |
| First | 49.76 | 7.35 | 12.56 | 27.37 | 1.49 | 50.63 |
| Second | 51.15 | 9.04 | 16.92 | 21.39 | ..... | 47.35 |
| Third | 50.68 | 9.50 | 16.48 | 21.15 | .19 | 47.57 |
| Average | 50.53 | 8.63 | 15.32 | 23.30 | ..... | 48.52 |
| One-half bloom stage: | | | | | | |
| First | 53.94 | 7.98 | 18.32 | 25.22 | ..... | 51.52 |
| Second | 49.76 | 8.94 | 15.48 | 23.14 | .18 | 47.96 |
| Third | 49.29 | 7.74 | 17.10 | 23.38 | .12 | 48.51 |
| Average | 51.00 | 8.22 | 16.97 | 23.91 | ..... | 49.33 |
| Three-fourths bloom stage: | | | | | | |
| First | 51.15 | 6.98 | 15.73 | 25.85 | .66 | 50.03 |
| Second | 47.90 | 6.82 | 16.40 | 21.15 | .01 | 44.40 |
| Third | 42.32 | 4.08 | 14.43 | 23.56 | ..... | 42.07 |
| Average | 47.12 | 5.96 | 15.52 | 23.52 | ..... | 45.50 |

Table 12 is a rearrangement of the data reported in Table 11 and aims to bring out differences between first, second, and third cuttings, rather than to emphasize the stages of maturity. The table shows that although first-cutting hay ranks only second in digestible crude protein, it excels the others in digestible nutrients. Second-cutting hay contains the highest average content of digestible crude protein. The average data when compared with that in Table 3 show a lower content of digestible crude protein and total digestible nutrients.

TABLE 12.—*Digestible nutrients per 100 pounds in first, second, and third cuttings of alfalfa hay when cut at the one-fourth, one-half and three-fourths stages of bloom*

[Averages of 1923 and 1924 experiments]

| Stage of bloom | Dry matter | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Total digestible nutrients |
|---|---|---|---|---|---|---|
| First cutting: | | | | | | |
| One-fourth | 49.76 | 7.35 | 12.56 | 27.37 | 1.49 | 50.63 |
| One-half | 53.94 | 7.98 | 18.32 | 25.22 | ..... | 51.52 |
| Three-fourths | 51.15 | 6.98 | 15.73 | 25.85 | .66 | 50.03 |
| Average | 51.62 | 7.44 | 15.54 | 26.15 | ..... | 50.73 |
| Second cutting: | | | | | | |
| One-fourth | 51.15 | 9.04 | 16.92 | 21.39 | ..... | 47.35 |
| One-half | 49.76 | 8.94 | 15.48 | 23.14 | .18 | 47.96 |
| Three-fourths | 47.90 | 6.82 | 16.40 | 21.15 | .01 | 44.40 |
| Average | 49.60 | 8.27 | 16.27 | 21.89 | ..... | 46.57 |
| Third cutting: | | | | | | |
| One-fourth | 50.68 | 9.50 | 16.48 | 21.15 | .19 | 47.57 |
| One-half | 49.29 | 7.74 | 17.10 | 23.38 | .12 | 48.51 |
| Three-fourths | 42.32 | 4.08 | 14.43 | 23.56 | ..... | 42.07 |
| Average | 47.33 | 7.11 | 16.00 | 22.70 | ..... | 46.05 |

The average figures for the third cutting are considerably lower because of the third cutting made at the three-fourths stage of bloom, which appears to be out of line with the rest of the hays.

The digestible nutrients per ton reported in Table 13 are of interest to the stockman who must buy hay in the open market. The table shows that first-cutting alfalfa contains the largest quantity of digestible nutrients per ton, while the second cutting is richer in digestible protein. The average results for the third cutting are considerably lowered, due to the inclusions of the late third cutting in the three-fourths stage of bloom. With this stage omitted from the averages, third-cutting hay compares favorably with second-cutting hay.

When the average of digestible nutrients at all the stages of first cutting are considered as 100 per cent, second cutting rates 92, and third cutting 91 per cent. (See Table 13.)

TABLE 13.—*The nutritive value of a ton of alfalfa hay as affected by the stage of maturity of the plant and the number of the cutting*

[Digestible nutrients in pounds per ton, basis of two years' results]

| Stage of bloom | Dry matter | Crude protein (N×6.25) | Crude fiber | Total digestible nutrients |
|---|---|---|---|---|
| First cutting: | | | | |
| One-fourth | 995 | 147 | 251 | 1,013 |
| One-half | 1,079 | 160 | 366 | 1,030 |
| Three-fourths | 1,023 | 139 | 315 | 1,001 |
| Average | 1,032 | 149 | 311 | 1,015 |
| Second cutting: | | | | |
| One-fourth | 1,023 | 181 | 338 | 947 |
| One-half | 995 | 179 | 320 | 959 |
| Three-fourths | 958 | 136 | 328 | 888 |
| Average | 992 | 165 | 329 | 931 |
| Third cutting: | | | | |
| One-fourth | 1,014 | 190 | 330 | 951 |
| One-half | 986 | 155 | 342 | 970 |
| Three-fourths | 846 | 82 | 289 | 841 |
| Average | 949 | 142 | 320 | 921 |

## YIELD OF DIGESTIBLE NUTRIENTS PER ACRE

At the Washington Irrigation Branch Station (*36*) yield records kept over a period of six years show that when alfalfa is cut in the three-fourths bloom stage a maximum tonnage of air-dried hay to the acre is secured. However, the one-half bloom stage is a close second, and is given preference, since cutting alfalfa at the three-fourths stage of bloom prolongs the growing season so that the third cutting is produced under less favorable growing conditions. As a result the yield of this last cutting is lowered considerably. Cutting at the one-fourth bloom stage seems impractical in view of the lower yields. The six-year average shows the yield per acre for all three cuttings to be 5.83, 6.71, and 6.86 tons at the one-fourth, one-half, and three-fourths stages of bloom, respectively.

On the basis of these yield records and the digestion experiments, the digestible nutrients per acre have been computed and are shown in Table 14.

TABLE 14.—*Digestible nutrients produced per acre of alfalfa by three cuttings at the one-fourth, one-half, and three-fourths stages of bloom*

[Based upon digestion trials with two years' study of hay samples, and six-year yield data]

| Cutting | Air-dried hay [a] | Digestible nutrients per acre | | | | | |
|---|---|---|---|---|---|---|---|
| | | Dry matter | Crude protein (N×6.25) | Crude fiber | Nitrogen-free extract | Ether extract | Total digestible nutrients |
| One-fourth bloom stage: | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* |
| First | 4,180 | 2,080 | 307 | 525 | 1,144 | 62 | 2,116 |
| Second | 4,000 | 2,046 | 362 | 677 | 856 | ---- | 1,894 |
| Third | 3,480 | 1,764 | 331 | 574 | 736 | 7 | 1,655 |
| Total | 11,660 | 5,890 | 1,000 | 1,776 | 2,736 | ---- | 5,665 |
| One-half bloom stage: | | | | | | | |
| First | 5,320 | 2,870 | 425 | 975 | 1,342 | ---- | 2,741 |
| Second | 4,460 | 2,219 | 309 | 600 | 1,032 | 8 | 2,139 |
| Third | 3,640 | 1,794 | 282 | 622 | 851 | 4 | 1,766 |
| Total | 13,420 | 6,883 | 1,106 | 2,287 | 3,225 | ---- | 6,646 |
| Three-quarter bloom stage: | | | | | | | |
| First | 5,800 | 2,967 | 405 | 912 | 1,499 | 38 | 2,902 |
| Second | 5,060 | 2,424 | 345 | 830 | 1,070 | 1 | 2,247 |
| Third | 2,860 | 1,210 | 117 | 413 | 674 | ---- | 1,203 |
| Total | 13,720 | 6,601 | 867 | 2,155 | 3,243 | ---- | 6,352 |

[a] Washington Bulletin 209 (*36, p. 14, Table 4*), recomputed in pounds.

The digestible crude protein per acre of alfalfa is quite variable and is influenced by the number of the cutting and the stage of maturity of the plant. Only 117 pounds of digestible crude protein was produced to the acre in the third cutting of alfalfa harvested at the three-fourths stage of bloom, while a maximum of 425 pounds was secured with the first cutting in the one-half bloom stage. The first cutting at the three-fourths bloom stage produced 405 pounds.

The yield of hay in pounds to the acre appears to have no relationship to its content of digestible crude protein, and in several instances the lower yields of hay contained a larger quantity of this valuable nutrient. The maximum total protein production to the acre, based on all three cuttings, is secured at the one-half bloom stage of matur-

ity, the yield being 1,106 pounds. At the one-fourth bloom stage of maturity all three cuttings produced 1,000 pounds. The difference is not great, but the one-half bloom stage is to be preferred because it also yields a greater quantity of total digestible nutrients.

The production of digestible nutrients to the acre is of especial interest, since recent studies with dairy cows (*29*) indicate that existing figures of total digestible nutrients of feeds are a very good measure of the relative value of such feeds as sources of nutritive energy under practical conditions.

The highest seasonal yield of total digestible nutrients was secured at the one-half bloom stage, the three-fourths bloom stage ranking second. From the standpoint of total yield of hay, yield of digestible nutrients, and yield of digestible crude protein, it seems impractical to cut alfalfa at the one-fourth stage of bloom under the conditions of irrigation farming in the Yakima Valley.

The first cutting of alfalfa at the three-fourths bloom stage yielded the maximum of total digestible nutrients. However, the third cutting at this stage yielded less than half as much as the first. Second-cutting hay produces more total digestible nutrients and digestible crude protein than the third cutting at all stages.

Table 14 shows that the one-half bloom stage of maturity of the alfalfa plant is to be preferred to the earlier and later stages in respect to the yield of the several digestible nutrients.

## YIELD OF CALCIUM AND PHOSPHORUS PER ACRE AND PER TON OF HAY

The calcium and phosphorus content in the three cuttings of alfalfa cut for hay in different stages of maturity is not only of general interest to the livestock man, but also to the hay grower concerned in knowing how much of these elements are removed from his soil when the hay crop is sold to be fed elsewhere.

TABLE 15.—*Yield of calcium and phosphorus per acre and per ton of hay by three cuttings of alfalfa cut at the one-fourth, one-half, and three-fourths stages of bloom*

| Cutting | Air-dried hay per acre | Yield per acre of— | | Yield per ton of— | |
|---|---|---|---|---|---|
| | | Calcium | Phosphorus | Calcium | Phosphorus |
| One-fourth bloom stage: | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* |
| First | 4,180 | 54.42 | 6.44 | 26.04 | 3.08 |
| Second | 4,000 | 47.12 | 7.60 | 23.56 | 3.80 |
| Third | 3,480 | 45.07 | 6.37 | 25.90 | 3.66 |
| Total | 11,660 | 146.61 | 20.41 | | |
| Average | | | | 25.17 | 3.51 |
| One-half bloom stage: | | | | | |
| First | 5,320 | 78.63 | 8.19 | 29.56 | 3.08 |
| Second | 4,460 | 57.71 | 8.03 | 25.88 | 3.60 |
| Third | 3,640 | 52.78 | 5.28 | 29.00 | 2.90 |
| Total | 13,420 | 189.12 | 21.50 | | |
| Average | | | | 28.15 | 3.19 |
| Three-fourths bloom stage: | | | | | |
| First | 5,800 | 80.50 | 8.82 | 27.76 | 3.04 |
| Second | 5,060 | 64.26 | 6.78 | 25.40 | 2.68 |
| Third | 2,860 | 36.49 | 2.86 | 25.52 | 2.00 |
| Total | 13,720 | 181.25 | 18.46 | | |
| Average | | | | 26.23 | 2.57 |

Table 15 shows that at the one-fourth bloom stage all three cuttings contained 146.61 pounds of calcium. Yields of 189.12 and 181.25 pounds at the one-half and three-fourths bloom stages were secured. From the standpoint of total calcium yield per acre the one-half bloom stage is recommended. This is true for phosphorus also. The third cutting made at the three-fourths stage of bloom is quite deficient in calcium and phosphorus in comparison with the other hays. The one-half bloom stage of maturity also shows up to best advantage when the calcium and phosphorus content per ton of hay is computed, as seen in Table 15.

The conclusion that the hay highest in calcium is the best for livestock feeding is not warranted. It is the hay from which most calcium is assimilated that is of most value in bone development and milk production; and maximum calcium assimilation seems to depend upon the presence of the antirachitic vitamin contained either in the hay itself or in feeds fed with the alfalfa hay. Furthermore, there seems to be a relationship between the care taken in curing the hay and the antirachitic factor. The utilization of calcium from the different hays was not studied. Calcium and phosphorus balance studies would be a valuable addition. However, in a properly constituted ration the alfalfa hay higher in calcium is to be preferred.

## NITROGEN METABOLISM STUDIES

In Table 16 the column headed "Nitrogen stored per 100 pounds live weight" is used for comparative purposes rather than the nitrogen balance column, since in the former the weight factor is eliminated. The column "Per cent of intake stored" is of value only when the intake of nitrogen per unit of live weight is constant for all lambs. Lambs utilize protein for tissue development in growth and for cellular expansion in fattening, and some protein is used for repair of protein tissue. When all these demands are supplied the excess nitrogen is excreted. For that reason the greater the intake of protein above this requirement, the lower is the percentage utilization of the protein.

Averages of nitrogen-balance studies with alfalfa-hay samples grown in 1923 and 1924 are reported in Table 16. The single year's data are not reported. Three determinations were made each year and the results averaged, so that each figure in the table represents the average of six determinations, except when otherwise specified.

The average of two years' data shows that if all three cuttings are made at the one-fourth bloom stage the largest utilization of nitrogen is secured. The half-bloom stage ranks second while the three-fourths bloom stage is last. The same is true in respect to the nitrogen stored per 100 pounds live weight, which affords a truer basis of comparison in cases in which quite striking differences may be noted. In this respect the results show that the nitrogen utilization of the hay from less mature plants is markedly higher than that from the more mature plants. It decreases progressively as the plants mature.

The purpose of the preceding discussion was to show in what manner the stage of maturity of the alfalfa plant affected the protein utilization when hays of varying degrees of maturity were fed to lambs. A rearrangement of the data with a view to comparing first, second, and third cuttings was made, and Table 17 contains these results.

TABLE 16.—*Average results of nitrogen metabolism studies with alfalfa hay fed to lambs at various stages of maturity*

[Hay was produced in 1923 and 1924. Totals are for average 10-day period]

| Cutting | Initial weight of lambs | Intake of nitrogen in feed per head | Outgo of nitrogen in feces per head | Outgo of nitrogen in urine per head | Balance of nitrogen | Nitrogen stored per 100 pounds live weight | Intake of nitrogen stored |
|---|---|---|---|---|---|---|---|
| One-fourth bloom stage: | *Pounds* | *Grams* | *Grams* | *Grams* | *Grams* | *Grams* | *Per cent* |
| First | 71.68 | 202.29 | 76.58 | 75.44 | 50.27 | 71.95 | 24.72 |
| Second | 84.00 | 241.49 | 78.77 | 113.28 | 49.41 | 58.97 | 20.41 |
| Third | 85.42 | 242.25 | 75.93 | 98.86 | * 73.22 | * 85.60 | 28.68 |
| Average | 80.37 | 228.67 | 77.09 | 95.86 | 57.03 | 72.17 | 24.60 |
| One-half bloom stage: | | | | | | | |
| First | 78.04 | 215.71 | 73.50 | 104.73 | 37.47 | 47.29 | 17.20 |
| Second | 82.54 | 239.69 | 74.98 | 89.08 | 75.63 | 93.06 | 31.77 |
| Third | 86.17 | 218.90 | 82.69 | 90.07 | 46.17 | 53.85 | 19.29 |
| Average | 82.25 | 224.77 | 77.06 | 94.63 | 53.09 | 64.73 | 22.75 |
| Three-fourths bloom stage: | | | | | | | |
| First | 78.67 | 202.24 | 77.29 | 79.62 | 45.34 | 57.26 | 22.32 |
| Second | 86.67 | 199.06 | 78.88 | 80.93 | 39.24 | 46.13 | 19.55 |
| Third | 88.08 | 157.74 | 87.02 | 43.71 | * 33.57 | * 39.67 | * 21.77 |
| Average | 84.47 | 186.35 | 81.06 | 68.08 | 39.38 | 47.48 | 21.22 |

* Average of 5 determinations. All other figures are averages of 6 determinations, 3 with hays of 1923 and 3 with hays of 1924.

TABLE 17.—*Average results of nitrogen metabolism studies with alfalfa hay prepared from plants at the one-fourth, one-half, and three-fourths stages of bloom, arranged according to cuttings*

[Hays produced in 1923 and 1924. Total for average 10-day period]

| Stage of bloom | Initial weight of lambs | Feed in take of nitrogen in feed per head | Outgo of nitrogen in feces per head | Outgo of nitrogen in urine per head | Balance of nitrogen | Nitrogen stored per 100 pounds live weight | Intake of nitrogen stored |
|---|---|---|---|---|---|---|---|
| First cutting: | *Pounds* | *Grams* | *Grams* | *Grams* | *Grams* | *Grams* | *Per cent* |
| One-fourth | 71.68 | 202.29 | 76.58 | 75.45 | 50.27 | 71.94 | 24.72 |
| One-half | 78.04 | 215.71 | 73.50 | 104.63 | 37.46 | 47.30 | 17.20 |
| Three-fourths | 78.67 | 202.24 | 77.29 | 79.62 | 45.34 | 57.26 | 22.31 |
| Average | 76.13 | 206.74 | 75.79 | 86.59 | 44.36 | 58.83 | 21.41 |
| Second cutting: | | | | | | | |
| One-fourth | 84.00 | 241.48 | 78.77 | 113.28 | 49.40 | 58.97 | 20.41 |
| One-half | 82.54 | 239.69 | 74.98 | 89.08 | 75.13 | 93.06 | 31.77 |
| Three-fourths | 86.67 | 199.06 | 78.88 | 80.93 | 39.24 | 46.13 | 19.55 |
| Average | 84.40 | 226.74 | 77.54 | 94.43 | 54.76 | 66.05 | 23.91 |
| Third cutting: | | | | | | | |
| One-fourth | 85.42 | 242.25 | 75.93 | 98.86 | 73.22 | 85.60 | 28.69 |
| One-half | 86.17 | 218.90 | 82.70 | 90.07 | 46.17 | 53.85 | 19.29 |
| Three-fourths | 88.08 | 157.75 | 87.01 | 43.70 | 33.58 | 39.06 | 21.77 |
| Average | 86.56 | 206.30 | 81.88 | 77.54 | 50.98 | 59.50 | 23.24 |

The average data for all first cuttings, made at the one-fourth, one-half, and three-fourths stages of bloom during two years shows a storage of 58.83 gm. of nitrogen each 10-day period per lamb and per 100 pounds live weight. The protein of second-cutting hay is even better utilized, 66.05 gm. of nitrogen being stored. Third-

cutting hay ranks intermediate with a storage of 59.50 gm. The same order pertains in respect to the "Balance of nitrogen" and "Intake of nitrogen stored."

## GENERAL SUMMARY AND CONCLUSIONS

The main purpose of this investigation was the determination of the chemical composition, digestibility, and protein utilization of first, second, and third cuttings of alfalfa. Each of these three cuttings was studied at the one-fourth, one-half, and three-fourths stages of maturity, and samples grown in 1923 and 1924 were fed to lambs averaging 80 pounds in weight.

Fifty-four digestion and nitrogen-metabolism experiments were carried on and from these studies the digestible nutrients in the various hays were computed, and the utilization of protein determined.

The protein content of alfalfa hay decreased and the fiber content was found to increase, as the plants matured. The third cutting at the three-fourths stage of bloom contained the least protein. Second cutting (average of all stages) contained the highest percentage of protein, followed by the third. First cutting ranked last.

The calcium content of alfalfa was found to be seven to eleven times greater than its phosphorus content. Plants cut at the one-half bloom stage contained more calcium than similar samples harvested at the one-fourth and three-fourths bloom stages. No relationship between maturity and phosphorus content was observed, nor was there any relationship between the number of the cutting and the phosphorus and calcium content of the hays.

The highest content of digestible crude protein and total digestible nutrients was secured from the three cuttings of hay harvested at the one-half bloom stage.

First-cutting alfalfa hay contained the largest percentage of digestible nutrients per ton followed by the second and third cuttings, respectively. Second-cutting hay contained the maximum of digestible crude protein per ton and third cutting the least.

The highest seasonal yield of total digestible nutrients and digestible crude protein to the acre was secured at the one-half bloom stage. In respect to protein, hay at the one-fourth bloom stage ranked second, while the three-fourths bloom stage ranked second in respect to total digestible nutrients.

With increasing maturity of the alfalfa plants a lower utilization of the crude protein was observed.

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